agronomy

MDPI

Article

Association Mapping for Quantitative Trait Loci Controlling Superoxide Dismutase, Flavonoids, Anthocyanins, Carotenoids, γ-Oryzanol and Antioxidant Activity in Rice

Ramakrushna Bastia [1,2,†], Elssa Pandit [1,3,†], Priyadarsini Sanghamitra [1], Saumya Ranjan Barik [1], Deepak Kumar Nayak [1], Auromira Sahoo [1], Arpita Moharana [1], Jitendriya Meher [1], Prasanta K. Dash [4,*], Reshmi Raj [1], Binod Kumar Jena [5], Kartik Chandra Pradhan [2], Devraj Lenka [2], Nabaneeta Basak [1], Srikanta Lenka [1] and Sharat Kumar Pradhan [1,*]

1 ICAR-National Rice Research Institute, Cuttack 753006, India
2 College of Agriculture, OUAT, Bhubaneswar 751003, India
3 Bioscience & Biotechnology Department, Fakir Mohan University, Balasore 756020, India
4 ICAR-National Institute for Plant Biotechnology, New Delhi 110012, India
5 KVK, OUAT, Rayagada 765022, India
* Correspondence: prasanta01@yahoo.com (P.K.D.); pradhancrri@gmail.com (S.K.P.)
† These authors contributed equally to this work.

Abstract: Antioxidant-rich rice is a cheaper way to solve stress-related disorders and other health benefits for the global rice-eating population. Five antioxidant traits, namely, superoxide dismutase, flavonoids, anthocyanins, γ-oryzanol and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) activity were mapped using a representative panel population through association mapping. Potential landraces carrying multiple antioxidant compounds were identified from the population. The population represented four genetic groups and correspondence for presence of antioxidants traits in each group was noticed. The population showed linkage disequilibrium for the studied traits based on the Fst values. A total of 14 significant marker–trait associations were detected for these antioxidant traits. The study validated the QTLs, *qANC3* and *qPAC12-2* for anthocyanin content and *qAC12* for ABTS activity will be useful in marker-assisted breeding. Eleven QTLs such as *qTAC1.1* and *qTAC5.1* controlling anthocyanin content, *qSOD1.1*, *qSOD5.1* and *qSOD10.1* for superoxide dismutase (SOD), *qTFC6.1*, *qTFC11.1* and *qTFC12.1* for total flavonoids content (TFC), *qOZ8.1* and *qOZ11.1* for γ-oryzanol (OZ) and *qAC11.1* for ABTS activity were detected as novel loci. Chromosomal locations on 11 at 45.3 cM regulating GO, TFC and TAC, and on the chromosome 12 at 101.8 cM controlling TAC and ABTS activity, respectively, were detected as antioxidant hotspots.

Keywords: ABTS; antioxidant activity; anthocyanins; association mapping; flavonoids; γ-oryzanol; superoxide dismutase

Citation: Bastia, R.; Pandit, E.; Sanghamitra, P.; Barik, S.R.; Nayak, D.K.; Sahoo, A.; Moharana, A.; Meher, J.; Dash, P.K.; Raj, R.; et al. Association Mapping for Quantitative Trait Loci Controlling Superoxide Dismutase, Flavonoids, Anthocyanins, Carotenoids, γ-Oryzanol and Antioxidant Activity in Rice. *Agronomy* **2022**, *12*, 3036. https://doi.org/10.3390/agronomy12123036

Academic Editors: Gyuhwa Chung and Muhammad Amjad Nawaz

Received: 24 August 2022
Accepted: 30 September 2022
Published: 30 November 2022

1. Introduction

Rice is a principal food for more than half of the global population. The crop is mostly produced and consumed in Asiatic countries. However, the majority of rice-consuming people are observed to suffer problems such as malnutrition, Fe and Zn deficiency, and oxidative stress-related health problems such as stroke, psoriasis, type II diabetes, heart diseases, obesity, cancers, dermatitis, and rheumatoid arthritis [1,2]. Antioxidants protect cells against free radicals, which may cause diseases in human. As the majority of the global population is dependent on rice, enriching the grains with Fe, Zn, and antioxidant compounds are the priority areas of rice research [3–7]. Consumption of rice rich in antioxidants is a better and cheaper option for combatting the stress-related disorders and gaining other health benefits [7]. Enhancing the nutraceutical value of the antioxidant compounds in rice though biofortification is the best and cheapest way of achieving health

benefits for the people in a country [8]. In recent years, consumption of wholegrain pigmented rice enriched with antioxidant compounds has been gaining popularity in developed and developing countries due to its health benefits of reducing the risk of many chronic diseases [9–11]. Thus, rice breeding programs need to focus on the development of nutrient-dense rice for which improvement of the antioxidant traits is a necessity to meet the nutritional quality standards. Therefore, locating the genes and QTLs regulating these antioxidants in rice grain is very important research to conduct before starting an improvement program for these traits.

Antioxidants are present in plants both in enzymatic and non-enzymatic forms. Enzymatic antioxidants are catalases, peroxidases, superoxide dismutases, glutathione and other proteins and non-enzymatic antioxidants include phenolic defense compounds (vitamin E, flavonoids, phenolic acids and other phenols); nitrogen compounds (alkaloids, amino acids and amines), carotenoids, and chlorophyll derivatives [12,13]. The enzymatic antioxidants protect the plant cell from damage caused by reactive oxygen species and act as a defense system for maintaining the structural and functional integrity of a cell by inhibiting the oxidative deterioration to macromolecules such as lipid, protein, and nucleic acid [13–15]. Hence, improvement of these traits in rice will lead to the development of better-quality rice. Non-enzymatic antioxidants such as phenolic acids, flavonoids, anthocyanins and proanthocyanidins, tocopherols and tocotrienols (vitamin E), and γ-oryzanol have beenreported to be higher in pigmented rice (rice with red, black, and purple pericarp). The antioxidants show impressive health benefits such as reducing oxidative stress and cholesterol levels in human body, lowering the chances of type II diabetes, obesity, cancer, etc. [16–18]. These antioxidant traits are complex traits, polygenic in nature and quantitatively inherited [19]. Understanding the genetic bases of these complex antioxidant traits and identification of major QTLs are essential for the improvement of these phytochemicals through molecular breeding to ameliorate the increasing nutrition problems of the rice-eating population and seed quality, as well.

Identification of QTLs/genes for higher carotenoid content and development of functional markers is slow in rice as reports of carotenoids are not available in rice [20]. Wide genetic variation for carotenoid content exists in rice. White rice accumulates a very small quantity of carotenoid [21,22]. Color-providing pigments, anthocyanidin and proanthocyanidin, are present in the pericarp and aleurone layer of rice grain. The proanthocyanidin content in rice imparts red color to rice pericarp is controlled by the interaction of *Rc* and *Rd* genes [23–25]. Whereas, anthocyanidins impart purple-black pericarp to rice grain which is controlled by two loci, *Pb* and *P* [26]. Two genes, dihydroflavonolreductase (*DFR*) and anthocyanin synthase (*ANS*), present on chromosome 1 regulate the anthocyanin content in rice seeds [27]. However, a recent study reported that A1 (*Kala1/Rd/OsDFR*) and C1alleles (*OsC1)* determine the purple color of grain, and the pattern of anthocyanin pigmentation in grain is determined by the allelic status of A1, C1, and S1 (*OsANS1*) [28]. *Kala 4/OsB2/Pb* gene was mainly responsible for black pigmentation of rice pericarp [29,30].

The genetic analyses for identification and fine mapping of genes and QTLs for pericarp pigmentation in rice have been published by many workers using various mapping populations [10,19,25,30,31]. However, few reports on QTL mapping are available for γ-oryzanol, total phenolics content (TPC), total flavonoids content (TFC), ABTS (Azinobis 3-ethyl benzothiazoline-6-sulfonic acid), and SOD (Super oxide dismutase) traits in rice. Flavonoids are the major class of phenolic compounds responsible for color in rice. Rice bran contains seven flavonoids, of which tricin is the key compound. The QTLs, *qPH-12*, *qFL-2-1* and *qAC-1*, control the phenolic content, flavonoid content, and antioxidant capacity, respectively, in rice [10,31–33]. A mapping study on γ-oryzanol content in rice was reported by Kato et al. [34]. In addition, recent reports indicated the possibility of marker–trait association for phenolics, carotenoids, anthocyanin, γ-oryzanol, and other antioxidant contents in rice [9,35]. In addition, the antioxidant traits are reported to be regulated by different pathways, *viz*. Phenylpropanoid biosyn-

thesis pathway for flavonoids [36,37]; Methylerythritol 4-phosphate (MEP) pathway for carotenoids [38,39]; Mevalonate (MVA) pathway [40]; chromogen activator and tissue-specific regulator (CAP) regulatory pathway [41]; Phenyl propanoid metabolic pathway [42], or Phenyl alanine pathway [43] for anthocyanins; Esterification of hydroxyl sterols for Gamma-oryzanols [42] and Mitogen activated protein (MAP) kinase pathway for superoxide dismutase [44], from which insights into molecular mechanisms of the traits are possible.

Association mapping based on linkage disequilibrium has emerged as a powerful alternative strategy for identifying genes or quantitative trait loci (QTL) for various complex traits in plants by analyzing natural variable population. The genetic diversity and structure of the population will be helpful for detecting marker–trait association which could be useful for trait enhancement in molecular breeding programs. In order to avoid spurious association of marker-phenotype in a population, population structure (Q) with relative kinship (K) analyses are essential to check the adequacy of the panel population composition for linkage disequilibrium (LD) mapping analyses [45,46]. Thus, association estimates based on both the models of Generalized linear model and Mixed linear model are considered appropriate for mapping complex traits that have been shown to perform better than other model analyses. Although several genes for these antioxidant traits have been reported, more genes/loci are still to be identified to explain the complex regulation of carotenoids, SOD, total anthocyanins, γ-oryzanols, TFC, and ABTS in rice grains. In the present study, we have mapped these six antioxidant traits through association mapping in a highly variable representative set of 120 rice population representing the landraces and cultivars (67 white and 53 red grain) from an original population of 270 germplasm lines using 136 rice microsatellite markers.

2. Materials and Methods

2.1. Seed Materials

The study material comprised of 270 genotypes (landraces and cultivars) of 121 white and 149 colored rice grains. The initial population was shortlisted on the basis of maturity duration (upto 135 days) and kernel color (red, black, purple, and white) from about 1000 germplasm lines. Seeds of these germplasm were collected from Gene bank, ICAR-National Rice Research Institute, Cuttack and were grown in the experimental plot of the Institute during wet season, 2019. The genotypes were grown in a randomized complete block design in three rows, each with a spacing of 20 × 15 cm, in two replications, by following recommended package and practices. Each replication is divided into 5 blocks by accommodating 54 germplasm lines in each block. Panicles from middle-row plants of each replication were harvested, sun dried for 4–5 days to reduce the moisture content to 11–12%, stored for three months to remove dormancy, and then used for estimation of superoxide dismutase, flavonoids, anthocyanins, carotenoids, γ-oryzanol and antioxidant activity. A representative panel population containing 120 germplasm lines was prepared from the original 270 germplasm lines (120 genotypes consisting of 67 white and 53 red grain rice). The panel population was raised during wet season, 2019 and 2020. The harvested seeds from both years were used for the estimation of antioxidant traits. The panel population (120) was used in the genotyping for association mapping of antioxidant traits (Table 1).

2.2. Phenotyping for the Antioxidant Traits

The seed samples were dehulled by the Satake rice huller, Japan and were ground into flour by a grinding machine (Glenmini grinder) and sieved through a 100-mesh-size sieve, and then stored at 4 °C. Analyses of all the traits were based on dry matter basis, except for carotenoid content, which was estimatedon a fresh-weight basis. Leaf samples from 10 days old seedling grown on aPetri dish at 30 °C were used for estimation of carotenoids (mg g^{-1}) by following the protocol of Davis [47]. Seed enzymatic antioxidant, super oxide dismutase (SOD: unit g^{-1}), was estimated as per the proce-

dure of Madamanchi et al. [48]. Non-enzymatic antioxidant, total anthocyanin content (TAC: mg 100 g^{-1}) was estimated by the procedure of Fuleki and Francis, [49]. Estimation of γ-Oryzanol (GO:mg 100 g^{-1}) was performed according to Bucci et al. [50] with minor modifications. Total flavonoids content (TFC) was estimated as per the procedure of Eberhardt et al. [51] and expressed as catechin equivalent (mg CEt 100 g^{-1}). Antioxidant activity, 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging was assayed by the modified protocol of Serpen et al. [52] and expressed as % inhibition.

Table 1. Mean values of carotenoids, SOD, TAC, GO, TFC, and ABTS antioxidants estimated from 120 genotypes present in the panel population.

Sl. No.	Kernel Color	Genotype/ Vernacular Name/Accession No.	Carotenoids	SOD	TAC	GO	TFC	ABTS
1	White	Ac. 5993	0.115	0.239	0.209	43.750	12.333	8.853
2	White	Ac. 6221	0.423	0.101	0.159	47.375	13.333	8.952
3	White	Ac. 6183	0.182	0.015	0.102	47.125	13.889	14.119
4	White	Ac. 6170	1.165	0.176	0.090	52.250	13.333	11.063
5	White	Ac. 6023	0.112	0.280	0.143	33.313	13.000	10.522
6	White	Ac. 6172	0.297	0.181	0.225	34.125	13.444	7.569
7	White	Ac. 6027	0.133	0.175	0.141	38.188	12.333	7.983
8	White	Ac. 6007	0.287	0.192	0.027	32.125	13.111	7.983
9	White	Ac. 9006	1.014	0.284	0.064	70.438	17.889	11.412
10	White	Ac. 9021	0.444	0.199	0.083	76.313	22.000	11.769
11	Red	Ac. 9028	0.776	0.216	0.250	87.500	45.556	36.976
12	White	Ac. 9030	0.686	0.150	0.123	39.563	18.667	11.555
13	White	Ac. 9035	0.262	0.196	0.117	49.500	17.667	11.698
14	Red	Ac. 9038	0.371	0.241	0.459	28.438	47.000	41.341
15	White	Ac. 9043	0.308	0.175	0.061	39.688	18.444	8.131
16	White	Ac. 9044A	0.713	0.221	0.048	49.938	17.556	15.906
17	Red	Ac. 20920	1.264	0.312	0.325	54.125	43.889	26.061
18	Red	Ac. 20907	0.919	0.308	0.551	64.750	52.444	26.501
19	White	Ac. 20845	1.257	0.265	0.102	61.250	18.889	6.442
20	Red	Ac. 20770	1.379	0.313	0.568	62.688	62.333	35.959
21	Red	Ac. 20627	1.164	0.245	0.451	93.375	45.333	22.694
22	White	Ac. 20686	0.968	0.290	0.073	43.188	21.778	4.539
23	White	Ac. 20664	0.828	0.256	0.070	51.938	19.778	7.028
24	Red	Ac. 20614	0.727	0.273	0.609	85.250	62.841	38.448
25	White	Jhagrikartik	0.080	0.209	0.167	39.688	15.222	10.623
26	White	Dadghani	0.411	0.206	0.130	51.875	16.000	12.606
27	White	Shayam	0.455	0.196	0.170	58.313	19.333	12.677
28	White	Basumati-B	0.091	0.177	0.124	55.750	20.667	15.935
29	Red	Bharati	0.108	0.235	0.442	41.250	35.667	33.669
30	White	Joha	0.094	0.248	0.155	41.688	17.000	11.402
31	Red	Adira-1	0.350	0.137	0.943	46.750	114.222	39.115
32	Red	Adira-2	0.511	0.094	0.901	54.313	80.111	38.316
33	Red	Adira-3	0.472	0.039	2.996	48.750	79.667	38.099

Table 1. *Cont.*

Sl. No.	Kernel Color	Genotype/ Vernacular Name/Accession No.	Carotenoids	SOD	TAC	GO	TFC	ABTS
34	Red	PK6	0.217	0.112	1.168	46.125	62.222	33.091
35	Red	Vachaw	0.388	0.078	1.568	47.563	54.111	39.317
36	Red	Kozhivalan	0.476	0.007	0.684	51.500	67.667	27.279
37	Red	Marathondi	0.479	0.059	0.501	45.188	45.556	35.626
38	Red	Ezhoml-2	0.234	0.035	0.801	46.688	85.667	33.512
39	Red	Jyothi	0.437	0.062	0.901	56.750	58.889	31.916
40	Red	Kantakapura	0.947	0.068	0.417	39.000	62.333	36.994
41	Red	Kantakaamala	1.202	0.116	0.451	34.875	60.111	31.503
42	Red	Kapanthi	0.989	0.177	0.451	10.813	41.444	41.757
43	White	Karpurkanti	1.052	0.155	0.079	44.625	18.333	12.645
44	Red	Kathidhan	0.087	0.143	0.601	25.750	35.222	28.107
45	Red	Kundadhan	0.489	0.008	0.876	30.063	56.556	39.595
46	Red	Champaeisiali	0.360	0.222	0.534	20.688	31.444	30.275
47	White	Latamahu	0.493	0.189	0.141	23.375	21.444	13.584
48	Red	Latachaunri	0.507	0.211	1.018	19.875	50.444	30.925
49	White	Ac. 10608	0.427	0.087	0.108	43.125	12.333	10.414
50	White	Ac. 10187	0.395	0.159	0.085	30.063	37.111	12.981
51	Red	Ac. 10162	0.259	0.192	0.526	45.000	81.333	32.397
52	White	Ac. 7282	0.136	0.047	0.084	37.063	20.667	10.335
53	White	Ac. 7269	0.119	0.005	0.204	43.938	14.111	10.189
54	White	Ac. 7134	0.418	0.144	0.118	46.438	17.333	6.841
55	White	Ac. 7008	0.913	0.011	0.078	42.438	22.444	9.534
56	White	Ac. 9093	0.357	0.215	0.061	45.750	17.000	11.270
57	White	Ac. 9090	0.255	0.221	0.079	48.438	16.667	10.556
58	White	Ac. 9076A	0.899	0.159	0.048	43.688	22.889	12.126
59	Red	Ac. 9065	0.353	0.176	0.359	44.875	61.778	30.485
60	Red	Ac. 9063	0.860	0.235	0.375	110.563	52.222	23.538
61	White	Ac. 9058	0.573	0.126	0.055	5.313	23.222	11.698
62	White	Ac. 9053A	0.154	0.159	0.053	42.000	17.333	9.415
63	Red	Ac. 9050	0.395	0.191	0.388	28.313	54.889	32.411
64	White	Ac. 9005	1.612	0.268	0.126	47.375	24.333	14.622
65	White	Ac. 20389	1.247	0.279	0.035	66.250	19.333	10.102
66	White	Ac. 20371	0.839	0.284	0.083	110.000	32.000	6.149
67	Red	Ac. 20423	0.713	0.182	0.434	46.625	53.000	33.031
68	White	Ac. 20362	0.811	0.312	0.077	68.750	19.222	10.688
69	White	Ac. 20328	1.331	0.312	0.078	67.500	22.000	6.076
70	White	Ac. 20317	0.870	0.332	0.102	79.063	23.444	10.542
71	Red	Ac. 20282	1.118	0.201	1.043	84.500	76.889	42.167
72	Red	Ac. 20246	1.083	0.279	2.846	67.875	69.333	41.947
73	Red	Ac. 20347	1.188	0.292	0.272	57.313	23.778	27.906

Table 1. *Cont.*

Sl. No.	Kernel Color	Genotype/ Vernacular Name/Accession No.	Carotenoids	SOD	TAC	GO	TFC	ABTS
74	White	Palinadhan-1	0.094	0.342	0.150	38.313	21.000	14.589
75	White	Chatuimuchi	0.525	0.322	0.120	49.875	18.778	16.714
76	White	Uttarbangalocal-9	0.098	0.296	0.060	51.500	18.333	15.439
77	White	Gochi	0.098	0.323	0.118	42.000	22.000	14.731
78	White	Sugandha-2	0.273	0.278	0.127	57.125	19.444	11.615
79	White	Jhingesal	0.423	0.209	0.163	39.750	19.000	13.456
80	Red	Cheruvirippu	0.315	0.114	0.676	37.313	89.667	37.205
81	Red	Mahamaga	0.343	0.187	0.584	38.000	40.778	33.861
82	White	Jaya	0.091	0.079	0.093	43.688	14.778	16.255
83	Red	D1	0.164	0.153	0.451	81.938	73.111	37.997
84	Red	Pk-21	0.269	0.169	0.568	40.000	44.222	32.964
85	White	Gandhakasala	0.066	0.250	0.129	66.750	17.000	13.353
86	Red	Sreyas	0.217	0.148	0.618	57.375	119.889	31.495
87	Red	Gondiachampeisiali	0.762	0.213	0.626	24.750	54.556	24.855
88	White	Chinamal	0.748	0.300	0.111	18.313	22.222	9.104
89	White	Magra	0.146	0.311	0.119	19.875	17.111	9.971
90	Red	Landi	1.380	0.142	0.918	28.000	63.111	29.480
91	White	Lalgundi	0.353	0.289	0.124	10.563	22.222	11.272
92	White	Balisaralaktimachi	0.234	0.253	0.116	18.750	39.111	11.922
93	White	Laxmibilash	0.289	0.191	0.211	40.813	18.667	12.139
94	Red	Kaniar	1.027	0.214	0.651	39.000	16.778	21.532
95	White	Kanakchampa	0.129	0.272	0.159	39.313	16.444	15.795
96	White	Magura-s	0.210	0.295	0.134	43.063	16.000	13.512
97	White	Ac. 44603	1.098	0.227	0.110	60.875	43.889	13.088
98	Red	Ac. 44585	0.693	0.188	0.918	61.000	80.111	38.705
99	White	Ac. 44598	1.938	0.124	0.224	59.313	28.889	11.618
100	Red	Ac. 44592	1.032	0.118	2.320	64.938	242.000	50.515
101	Red	Ac. 44646	1.025	0.251	10.407	63.938	316.889	58.750
102	White	Ac. 44604	1.259	0.203	0.149	60.313	28.889	13.015
103	White	Ac. 44597	1.735	0.075	0.116	54.875	40.654	13.015
104	White	Ac. 44638	0.801	0.161	0.104	77.250	55.667	9.559
105	Red	Ac. 44595	1.014	0.145	6.618	66.500	334.111	69.412
106	Red	Ac. 44588	0.910	0.223	1.302	59.750	227.778	50.368
107	Red	Ac. 44591	1.158	0.206	0.818	47.188	124.111	35.147
108	Red	Ac. 44594	0.986	0.191	3.388	60.563	183.222	35.735
109	Red	Ac. 43737	0.136	0.295	11.934	37.375	230.222	48.544
110	White	Ac. 43660	1.197	0.292	0.220	41.250	26.778	12.955
111	White	Ac. 43732	0.665	0.257	0.079	31.063	33.778	35.239
112	White	Ac. 43661	0.164	0.281	0.107	43.000	50.778	24.600
113	Red	Ac. 43738	0.164	0.274	11.274	47.500	246.000	53.566

Table 1. *Cont.*

Sl. No.	Kernel Color	Genotype/ Vernacular Name/Accession No.	Carotenoids	SOD	TAC	GO	TFC	ABTS
114	White	Ac. 43669	1.028	0.243	0.115	55.063	31.505	40.175
115	White	Ac. 43663	0.154	0.269	0.217	40.625	62.667	15.429
116	Red	Ac. 43658	0.325	0.269	19.796	38.688	79.778	52.475
117	White	Ac. 43662	0.112	0.258	0.079	36.375	66.222	13.028
118	Red	Ac. 43670	0.115	0.282	28.375	56.813	358.444	81.441
119	White	Ac. 43675	0.168	0.238	0.115	40.875	24.444	32.678
120	Red	Ac. 43676	0.161	0.186	10.280	34.188	226.333	46.288
Mean			0.586	0.200	1.924	48.209	61.059	20.678
CV			12.25	3.100	12.800	1.810	6.700	6.200
LSD5%			0.174	0.0582	0.389	3.523	7.833	2.421

Carotenoids (mg g^{-1}); SOD: super oxide dismutase (unit g^{-11}); TAC: total anthocyanin content (mg 100 g^{-11}); GO: ga γ-oryzanol (mg 100 g^{-11}); TFC: Total flavonoids content (mg catechin or CEt 100 g^{-11}) and ABTS: 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (% inhibition).

2.3. Statistical Analysis

Cropstat software7.0 developed by IRRI was used for analysis of variance (ANOVA) for each trait and for the estimation of mean, range, and coefficient of variation (CV%). Pearson's correlation coefficients were analyzed to identify the relationship among the various antioxidant traits, based on the mean values of the 120 genotypes and presented in a correlation matrix heatmap by using PAST 4.03 software (Oyvind Hammer). The germplasm lines were classified into five groups as very high, high, medium, low, and very low categories based on the mean values of the antioxidant traits.

2.4. Genomic DNA Isolation, PCR Analysis, and Selection of SSR Markers

The genomic DNA was isolated from 15-day-old seedlings of the germplasm lines by adopting CTAB method [53]. A total of 136 SSR (simple sequence repeat) markers were selected from the database (http://gramene.org/, accessed on 24 August 2022) available in the public domain and used for genotyping of the panel population (Supplementary Table S5). The DNA fragments were resolved in gel electrophoresis for quantification of the isolated DNA. PCR analysis was performed using the markers selected based on positions covering all the chromosomes to illustrate the diversity and to identify the polymorphic loci among the 120 rice germplasm lines (Table 1). The conditions of reaction were set to initial denaturation step (2 min, 95 °C), followed by 35 cycles of denaturation (30 s, 95 °C) and annealing/extension (30 min, 55 °C), extension (2 min, 72 °C), final extension (5 min, 72 °C) and store at 4 °C (infinity). The PCR products were electrophoresed using 3% agarose gel containing 0.80 g mL^{-1} ethidium bromide and 50 bp DNA ladder was used to determine the size of amplicons. The gel was run for 4 h at 2.5 V cm^{-1} and photographed using a Gel Documentation System (Syngene). Earlier publications of molecular analysis were followed for DNA isolation, electrophoresis, and imaging techniques [54–57].

2.5. Molecular Data Analysis

Presence or absence of amplified products obtained on the basis genotype-primer combination was used to score the data. A binary data matrix was used as discrete variables for the entry of our result data. The parameters namely number of alleles (N), major allele frequency (A), polymorphic information content (PIC), observed heterozygosity (H), and gene diversity (GD) for each SSR locus were analyzed by using, 'Power Marker Version3.25' software [57]. A Bayesian model-based clustering approach STRUCTURE 2.3.6 software

was used to analyze genetic data and obtain population structure [58]. STRUCTURE software was run with K-values varying from 1 to 10, with 10 iterations for each K value to derive the ideal number of groups. A high throughput parameter set of burn-in period of the 150,000 followed by 150,000 Markov Chain Monte Carlo (MCMC) replications was adapted during the running period. The highest value of ΔK was obtained from the Evanno table used to detect the subpopulation groups from the panel of populations in the next step. The maximal value of L (K) was identified using the exact number of sub-populations. The model choice criterion to detect the most probable value of K was ΔK, an ad-hoc quantity related to the second-order change of the log probability of data with respect to the number of clusters inferred by STRUCTURE [59]. For estimation of the ΔK-value as a function of K showing a clear peak, the optimal K-value Structure Harvester was used [60]. The principal coordinate analysis of all the genotypes and unweighted neighbor joining unrooted tree for NEI coefficient dissimilarity index [61] with bootstrap value of 1000 were obtained by using DARwin5 software [62]. Analysis of molecular variance (AMOVA) using GenAlEx 6.5 software was used to estimate the presence of molecular variance across the whole population, within a population and between the sub-population structures (F_{IT}, F_{IS}, F_{ST}) calculated by the deviation from Hardy–Weinberg expectation. The procedures followed in earlier publications were adopted for molecular data analysis [63,64].

To analyze the marker–trait association for mapping study of the seed antioxidant traits in rice, the software "TASSEL 5.0" was used. General linear model and Mixed linear model in TASSEL 5.0 were used to calculate the genetic association between the phenotypic traits, and molecular markers were adopted as per Bradbury et al. [65]. By considering the significant *p*-value and r^2 value, convincing associated markers were identified. The associations of markers were further confirmed by the Q-Q plot generated by the software. Linkage disequilibrium plot was obtained using LD measured r^2, between pairs of markers plotted against the distance between the pair. Additionally, the accuracy of the marker–trait association was established by estimating the FDR adjusted *p*-values (q-values) using R software as described in the earlier publications [9,35,46].

3. Results

3.1. Phenotyping of the Population for the Six Antioxidant Traits

A total of five antioxidant compounds and one antioxidant enzyme, *viz.*, superoxide dismutase, flavonoids, anthocyanins, carotenoids, γ-oryzanol, and ABTS, were estimated from the 270 germplasm lines during wet season, 2019 (Supplementary Table S1). Wide genetic variation was observed for the six antioxidant traits in the germplasm lines. The genotypes were classified into five groups based on the phenotyping results of each compounds (Figure 1). The frequency distribution of germplasm lines showed various groups or populations for each compound and enzyme (Figure 1). A panel population was prepared by selecting 120 genotypes which represented each group and trait from the original population of 270 germplasm lines (Table 1; Figure 2). The mean estimates of six antioxidant traits obtained from the representative panel population showed wide variation among the genotypes for each trait (Table 1). Very high values of carotenoid content were found in germplasm lines Ac. 44598, Ac. 44597, and Ac. 9005. Additionally, very high TAC content was estimated from the lines Ac. 43670, Ac. 43660, and Ac. 43675. Germplasm lines namely Ac. 9063, Ac. 20371, and Ac. 20627 showed very high level of γ-oryzanol content in the seeds. Good donor lines were identified carrying very high TFC content, *viz.*, Ac. 43670, Ac. 43660, Ac. 44646, Ac. 44592, Ac. 44595, Ac. 43737, Ac. 43738, and Ac. 43676. The SOD level was found very high in the seeds of germplasm lines such as Ac. 20317, Palinadhan-1, Ac. 20362, Ac. 20328, Gochi, Chatuimuchi, Ac. 20770, Ac. 20920, Ac. 20907, Magra, and Chinamal. The potential donors identified for exhibiting very high level of ABTS were Ac. 44592, Ac. 43670, Ac. 4460, Ac. 44595, Ac. 44588, Ac. 43660, Ac. 43738, and Ac. 43732. However, germplasm lines (Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669) were identified for possessing a higher level of more than three antioxidant traits.

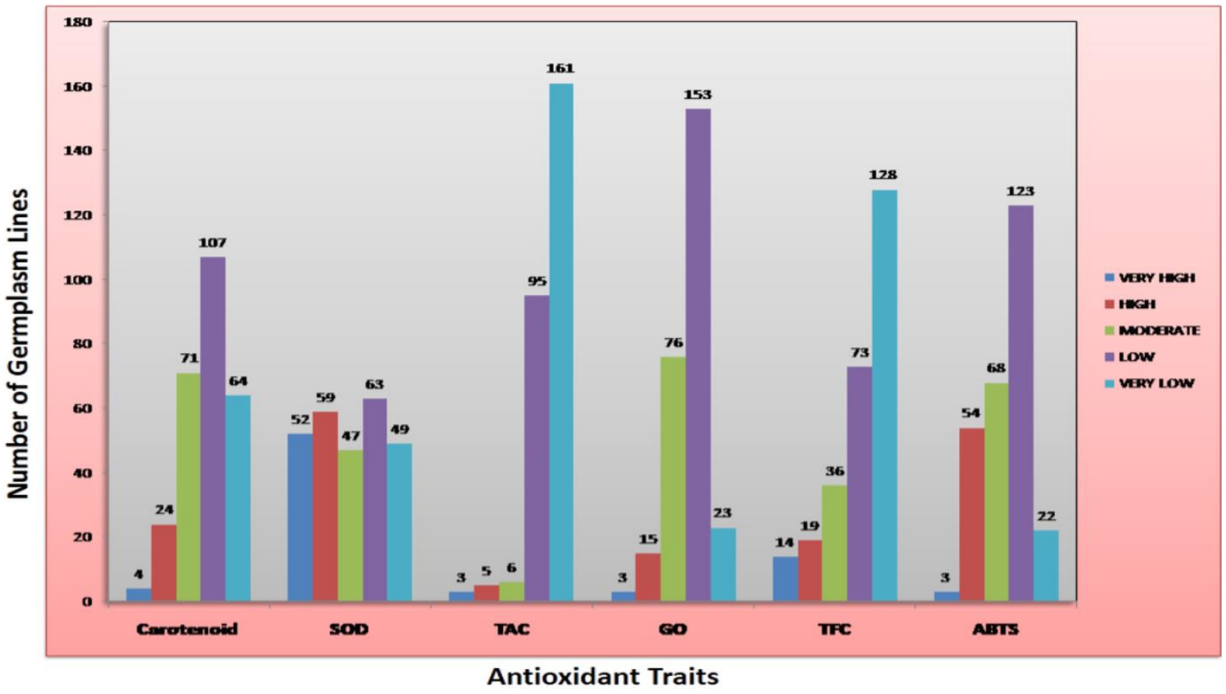

Figure 1. Frequency distribution of germplasm lines for each of the studied antioxidant traits, namely, carotenoids, superoxide dismutase, anthocyanins, γ-oryzanol, flavonoids, and ABTS present in the shortlisted 270 germplasm lines.

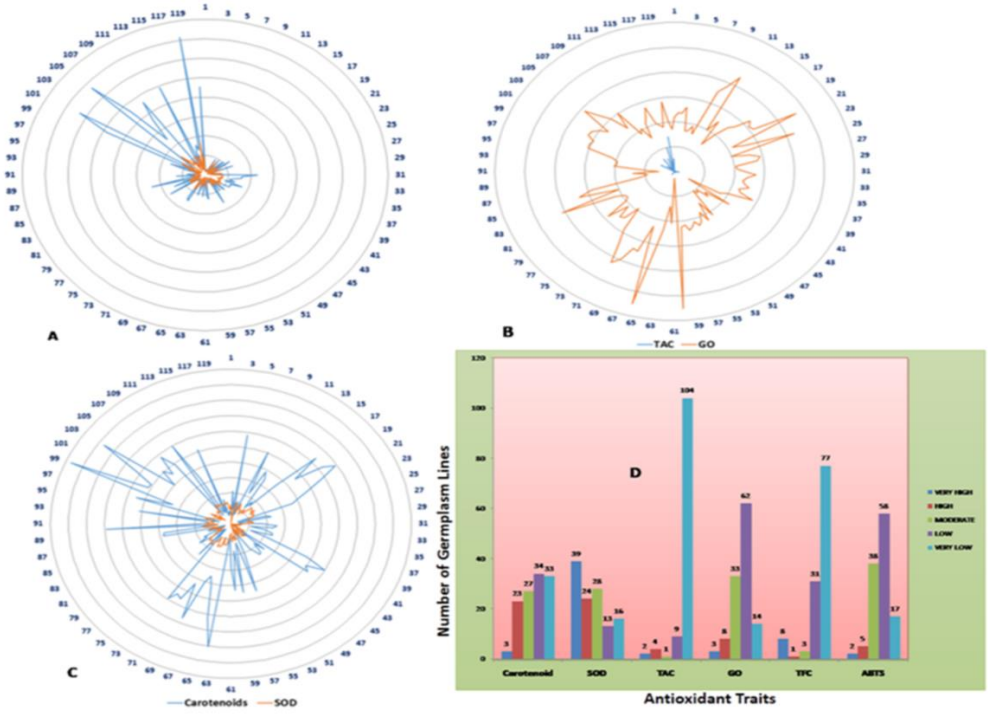

Figure 2. Estimate of 6 antioxidant traits in the 120 genotypes and their frequency distribution in the panel population. (**A**) Spider graph showing TFC content and ABTS activity. (**B**) TAC andγ-oryzanol content; (**C**) Carotenoid and SOD content; (**D**) Frequency distribution of germplasm lines for carotenoids, superoxide dismutase, anthocyanins, γ-oryzanol, flavonoids, and ABTS in the panel population.

3.2. Genotype-by-Trait Biplot Analysis for the Six Antioxidant Traits in the Germplasm Lines

The scatter diagram was plotted using the first two principal components to generate genotype-by-trait biplot graph for the six antioxidant traits in the 120 germplasm lines present in the panel (Figure 3). The first and second principal components showed 68.3 and 19.8%of the total variability with Eigen values of 8064 and 2342, respectively (Supplementary Figure S1). The compound, γ-oryzanol content contributed maximum diversity, followed by TFC and ABTS, among the six antioxidant traits estimated from the genotypes present in the panel (Figure 3). The scattering pattern of the germplasm lines in the four quadrants indicated that genotypes containing high estimates of the studied antioxidants are placed in quadrants I (top right) and II (bottom right). Higher estimates of the antioxidant traits with multiple compounds containing genotypes have been encircled in the figure (Figure 3). The top right (quadrant I) and bottom right (quadrant II) accommodated the majority of the genotypes containing high estimates of the antioxidant traits. The quadrant III (bottom left) kept most of the germplasm lines as moderate in the studied antioxidant traits, while the 4thquadrant (top left) accommodated the majority of poor germplasm lines for the antioxidants (Figure 3).

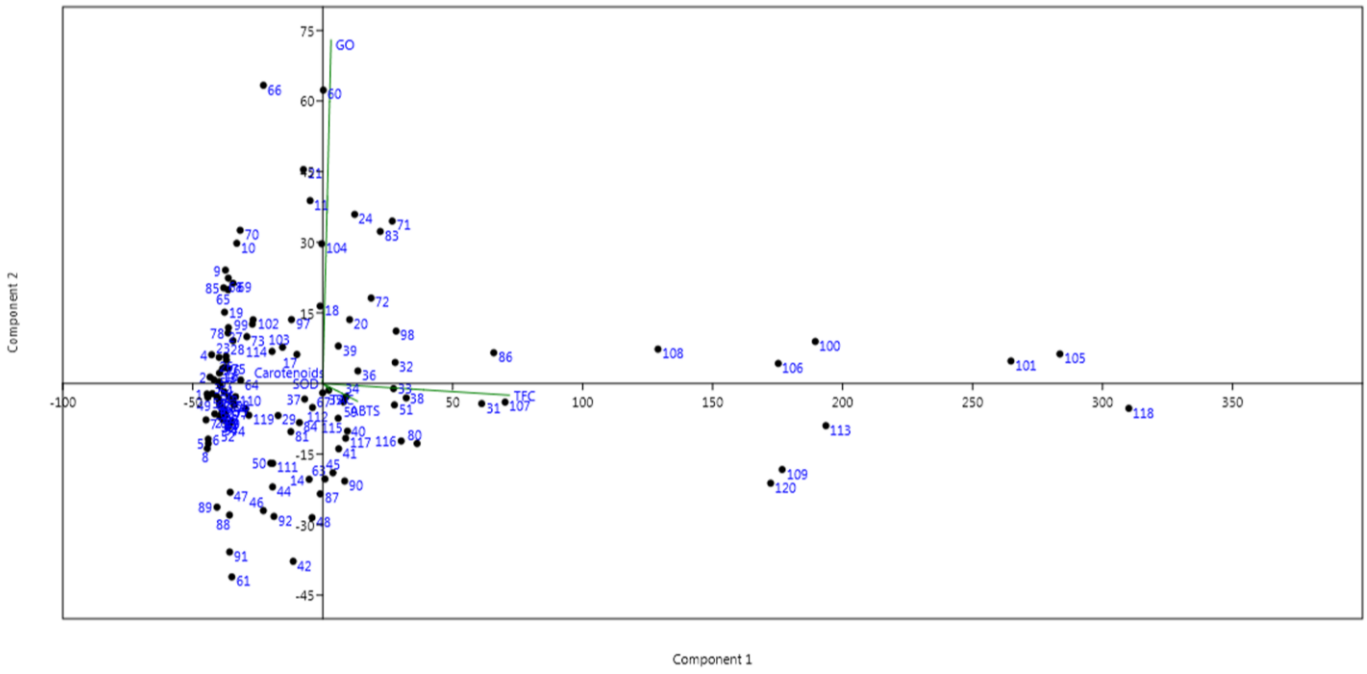

Figure 3. Genotype-by-trait biplot diagram showing 120 germplasm lines in two PCs for 6 antioxidant traits.

3.3. Nature of Association among the Antioxidant Traits

The association study provides information for correlation among the traits in which the correlated complex traits are useful in improvement programs. The association among the six antioxidant traits revealed a strong positive correlation ($r \geq 0.7$) of TAC with TFC and TFC with ABTS. Moderate positive correlation (r 0.5–0.7) of TAC with ABTS and a weak positive correlation ($r < 0.5$) were observed for carotenoid with γ-oryzanol content (Figure 4). These antioxidant traits positively or negatively correlated may be controlled by the closely linked genes or because they might be structurally related. Therefore, a variety that accumulates high concentrations of one antioxidant may also contain alarger quantity of other correlated antioxidants.

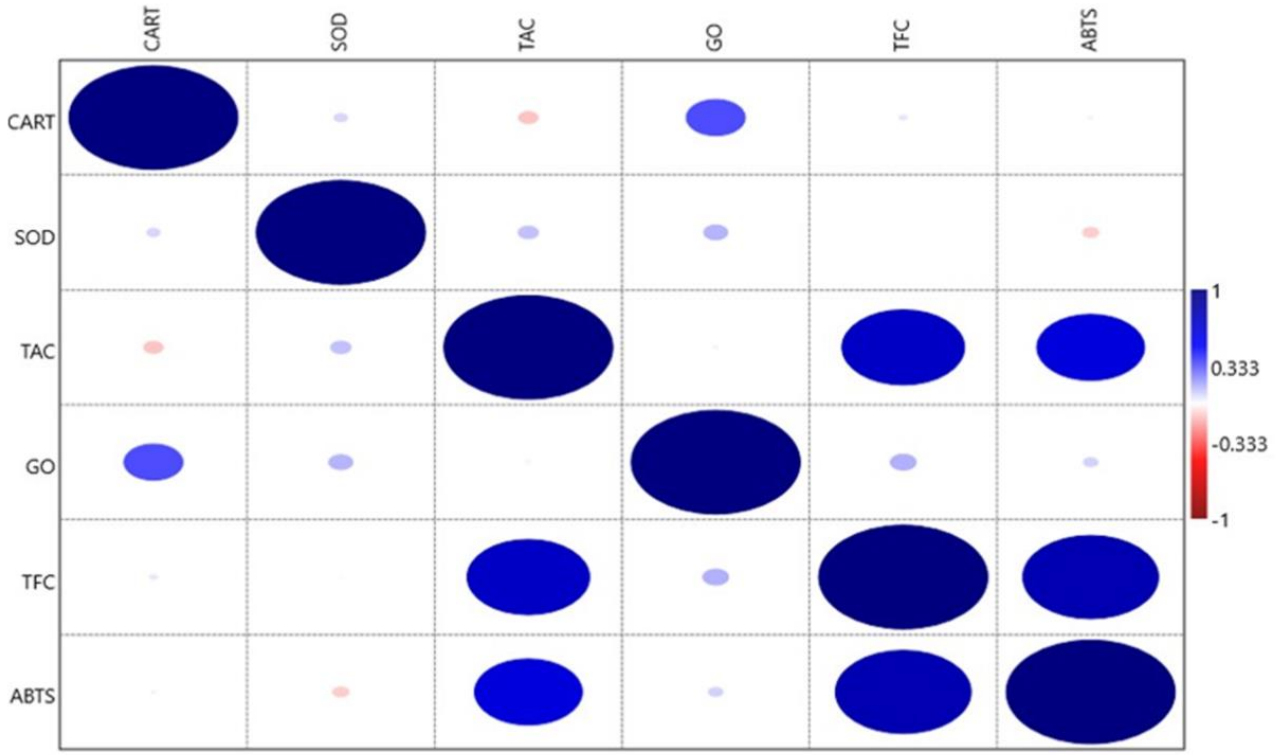

Figure 4. Heat map showing Pearson's correlation coefficients for 6 antioxidant traits. Significant correlations are colored either in red (negative) or blue (positive). Shades of blue indicate increasing positive correlation coefficient; shades of red indicate increasing negative correlation coefficient. CART: Carotenoids (mg g $^{-1}$); SOD: Super oxide dismutase (unit g^{-1}); TAC: Total anthocyanin content (mg 100 g^{-1}); GO: γ-oryzanol (mg 100 g^{-1}); TFC: Total flavonoids content (mg catechin or CEt 100 g^{-1}); ABTS: 2, 2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (% inhibition).

3.4. Genetic Diversity Parameters Analysis

The studied panel population exhibiting wide genetic variation in 120 germplasm lines for the six antioxidant traits was genotyped using 136 SSR markers. The genetic diversity parameters estimated from the panel population are depicted in Table 2. Genotyping results showed a total of 508 markers' alleles from the population, exhibiting mean alleles of 3.74 per locus. The number of alleles per locus ranged from 2 to 7 per marker. The largest numbers of alleles were produced by the marker RM493 in the studied panel population. The measure for the variation by a marker in the population was analyzed by the availability of major allele frequency parameter. The average major allele frequency linked to the polymorphic markers was computed to be 0.561, which showed a range of 0.279 (RM8044) and 0.925 (RM6054) (Table 2). The informativeness of a genetic marker is estimated by the PIC value. It ranged from 0.137 (RM6045 and 6054) to 0.787 (RM493) with an average value of 0.496. In case of low predicted heterozygosity of alleles in a population, the population may be shifting towards inbreeding for that trait. If it is higher than the predicted heterozygosity, that may be the effect of mixing of two genetic populations. Here, the observed mean heterozygosity (Ho) in the population was 0.116 which varied from 0.00 to 0.958 (RM3735). Twenty marker loci showed 0.00 Ho value in the panel population. The gene diversity (He), which gives a measure of genetic diversity in the panel population, ranged from 0.142 (RM6054) to 0.813 (RM493) with a mean value of 0.555.

Table 2. Estimation of genetic diversity parameters based on 136 SSR marker loci in a panel containing 120 rice germplasm lines.

Sl. No	Marker	No. of Alleles	Range of Amplicon (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC	Inbreeding Coefficient (f)
1	RM5310	4	140–190	0.783	0.367	0.033	0.343	0.910
2	RM582	4	210–245	0.708	0.466	0.033	0.433	0.929
3	RM13335	4	160–180	0.563	0.532	0.008	0.435	0.984
4	RM6275	4	140–160	0.721	0.447	0.058	0.411	0.870
5	RM50	4	190–205	0.400	0.689	0.025	0.630	0.964
6	RM85	4	80–110	0.413	0.675	0.125	0.615	0.816
7	RM222	4	210–250	0.629	0.557	0.025	0.519	0.956
8	RM247	5	140–200	0.500	0.597	0.067	0.519	0.889
9	RM328	3	185–200	0.567	0.580	0.000	0.513	1.000
10	RM337	6	155–400	0.446	0.668	0.117	0.612	0.827
11	RM340	5	100–220	0.713	0.454	0.100	0.415	0.781
12	RM470	5	60–140	0.463	0.690	0.833	0.644	−0.203
13	RM472	3	290–410	0.513	0.508	0.092	0.387	0.821
14	RM506	3	120–130	0.683	0.459	0.133	0.390	0.712
15	RM1812	3	130–140	0.442	0.607	0.000	0.523	1.000
16	RM3701	4	160–260	0.675	0.484	0.492	0.428	−0.012
17	RM6947	3	150–160	0.883	0.212	0.000	0.199	1.000
18	RM14978	3	240–250	0.417	0.639	0.000	0.563	1.000
19	RM18776	3	175–200	0.846	0.267	0.025	0.242	0.907
20	RM22034	3	75–85	0.917	0.155	0.000	0.147	1.000
21	RM24161	4	270–290	0.542	0.612	0.117	0.552	0.811
22	RM223	5	110–170	0.654	0.536	0.058	0.504	0.892
23	RM440	5	160–210	0.408	0.689	0.258	0.634	0.628
24	RM201	4	150–160	0.467	0.645	0.217	0.581	0.666
25	RM216	4	145–160	0.513	0.639	0.125	0.583	0.806
26	RM258	3	140–150	0.383	0.652	0.000	0.576	1.000
27	RM286	4	100–130	0.471	0.632	0.100	0.562	0.843
28	RM3735	4	135–500	0.333	0.725	0.958	0.674	−0.318
29	RM1347	3	100–110	0.517	0.566	0.000	0.475	1.000
30	RM7571	3	130–140	0.713	0.433	0.008	0.373	0.981
31	RM14723	4	220–250	0.492	0.643	0.200	0.581	0.691
32	RM103	3	255–330	0.492	0.559	0.767	0.461	−0.369
33	RM315	3	135–140	0.867	0.235	0.000	0.214	1.000
34	RM225	3	135–150	0.525	0.547	0.183	0.449	0.667
35	RM486	3	130–140	0.654	0.469	0.108	0.380	0.770
36	RM256	3	110–150	0.721	0.411	0.058	0.339	0.859
37	RM1113	3	150–180	0.671	0.457	0.058	0.373	0.873
38	RM3423	3	125–140	0.500	0.575	0.000	0.484	1.000

Table 2. *Cont.*

Sl. No	Marker	No. of Alleles	Range of Amplicon (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC	Inbreeding Coefficient (f)
39	RM6100	3	170–180	0.442	0.643	0.033	0.569	0.949
40	RM590	3	140–150	0.725	0.431	0.067	0.384	0.846
41	RM5793	3	115–130	0.633	0.525	0.017	0.464	0.969
42	RM405	3	100–110	0.675	0.491	0.000	0.441	1.000
43	RM547	5	190–300	0.471	0.573	0.167	0.481	0.711
44	RM7364	5	180–250	0.621	0.573	0.167	0.541	0.711
45	RM205	3	130–180	0.621	0.532	0.025	0.467	0.953
46	RM167	4	130–180	0.704	0.463	0.100	0.421	0.786
47	RM229	5	120–140	0.358	0.710	0.133	0.657	0.814
48	RM20A	3	230–240	0.625	0.533	0.017	0.472	0.969
49	RM235	5	100–145	0.396	0.719	0.175	0.671	0.758
50	RM7003	4	100–110	0.667	0.502	0.083	0.453	0.835
51	RM5436	4	155–190	0.442	0.621	0.058	0.545	0.907
52	RM25181	5	130–160	0.379	0.710	0.167	0.660	0.767
53	RM469	3	100–110	0.621	0.524	0.042	0.452	0.921
54	RM6547	3	155–165	0.867	0.240	0.017	0.226	0.931
55	RM152	4	145–155	0.508	0.628	0.017	0.565	0.974
56	RM148	2	140–150	0.675	0.439	0.083	0.342	0.812
57	RM421	3	250–260	0.458	0.631	0.000	0.555	1.000
58	RM2634	3	100–120	0.379	0.658	0.025	0.584	0.962
59	RM248	4	75–115	0.346	0.732	0.117	0.684	0.842
60	RM7179	5	50–250	0.325	0.765	0.358	0.727	0.535
61	RM215	3	155–165	0.617	0.491	0.017	0.392	0.966
62	RM324	4	220–260	0.542	0.635	0.158	0.590	0.753
63	RM317	3	150–160	0.725	0.403	0.000	0.328	1.000
64	RM174	3	230–270	0.508	0.621	0.067	0.551	0.893
65	RM556	3	190–210	0.842	0.279	0.033	0.260	0.881
66	RM257	4	130–155	0.408	0.663	0.233	0.595	0.651
67	RM502	3	260–265	0.808	0.318	0.000	0.281	1.000
68	RM331	4	95–115	0.483	0.664	0.058	0.611	0.913
69	RM403	4	110–130	0.596	0.570	0.083	0.515	0.855
70	RM309	3	180–190	0.696	0.460	0.025	0.405	0.946
71	RM6641	3	140–145	0.567	0.583	0.000	0.517	1.000
72	RM3	3	110–120	0.383	0.663	0.033	0.589	0.950
73	RM594	3	300–320	0.588	0.558	0.008	0.488	0.985
74	RM3392	4	160–180	0.504	0.615	0.108	0.545	0.825
75	RM1278	3	135–150	0.783	0.361	0.067	0.329	0.817
76	RM168	3	95–125	0.625	0.510	0.150	0.431	0.708
77	RM3375	3	190–200	0.567	0.576	0.033	0.506	0.943

Table 2. *Cont.*

Sl. No	Marker	No. of Alleles	Range of Amplicon (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC	Inbreeding Coefficient (f)
78	RM282	3	140–150	0.725	0.436	0.000	0.395	1.000
79	RM26632	4	450–550	0.363	0.701	0.158	0.644	0.776
80	RM1341	3	170–190	0.613	0.529	0.025	0.455	0.953
81	RM4112	3	160–170	0.488	0.623	0.158	0.549	0.748
82	RM20377	4	300–380	0.771	0.369	0.067	0.326	0.821
83	RM210	5	130–180	0.363	0.734	0.700	0.687	0.051
84	RM218	4	130–160	0.583	0.585	0.033	0.531	0.943
85	RM494	5	130–180	0.383	0.717	0.025	0.670	0.965
86	RM336	5	105–160	0.383	0.711	0.092	0.661	0.872
87	RM3475	4	135–160	0.450	0.656	0.042	0.591	0.937
88	RM480	4	190–210	0.538	0.618	0.025	0.561	0.960
89	RM566	4	150–200	0.433	0.656	0.017	0.591	0.975
90	RM11701	3	210–230	0.642	0.471	0.000	0.375	1.000
91	RM220	6	85–130	0.358	0.745	0.183	0.703	0.756
92	RM488	6	155–200	0.321	0.750	0.192	0.708	0.746
93	RM6374	6	130–160	0.338	0.771	0.075	0.737	0.904
94	RM233	5	130–160	0.350	0.727	0.233	0.680	0.681
95	RM112	3	130–135	0.875	0.222	0.000	0.204	1.000
96	RM13600	4	105–130	0.479	0.662	0.100	0.607	0.850
97	RM495	3	145–165	0.600	0.560	0.033	0.499	0.941
98	RM493	7	180–250	0.283	0.813	0.558	0.787	0.317
99	RM444	5	180–240	0.321	0.773	0.158	0.737	0.797
100	RM468	3	210–220	0.771	0.379	0.025	0.346	0.935
101	RM6054	3	120–130	0.925	0.142	0.017	0.137	0.883
102	RM509	3	165–170	0.758	0.395	0.000	0.360	1.000
103	RM5638	6	190–240	0.613	0.587	0.133	0.558	0.775
104	RM8044	6	240–300	0.279	0.761	0.233	0.721	0.695
105	RM8271	5	180–250	0.404	0.723	0.133	0.679	0.817
106	RM171	4	380–420	0.517	0.633	0.058	0.575	0.909
107	RM16686	3	90–100	0.417	0.655	0.000	0.581	1.000
108	RM434	4	250–280	0.567	0.595	0.025	0.537	0.958
109	RM6091	4	70–80	0.817	0.318	0.000	0.299	1.000
110	RM209	4	145–175	0.542	0.612	0.000	0.552	1.000
111	RM245	4	145–155	0.583	0.577	0.000	0.518	1.000
112	RM1089	4	210–260	0.417	0.637	0.067	0.565	0.896
113	RM228	4	110–170	0.625	0.544	0.192	0.491	0.650
114	RM401	3	250–300	0.754	0.398	0.058	0.360	0.855
115	RM11	3	140–160	0.463	0.590	0.008	0.502	0.986
116	RM3351	3	170–190	0.583	0.517	0.000	0.420	1.000

Table 2. *Cont.*

Sl. No	Marker	No. of Alleles	Range of Amplicon (bp)	Major Allele Frequency	Gene Diversity	Heterozygosity	PIC	Inbreeding Coefficient (f)
117	RM5749	3	130–160	0.588	0.504	0.025	0.400	0.951
118	RM335	2	100–110	0.721	0.402	0.075	0.321	0.815
119	RM144	3	200–210	0.588	0.516	0.158	0.419	0.695
120	RM300	3	125–145	0.867	0.238	0.017	0.221	0.930
121	RM1132	4	90–125	0.358	0.724	0.033	0.674	0.954
122	RM400	4	210–260	0.367	0.717	0.467	0.665	0.353
123	RM471	3	100–120	0.800	0.338	0.000	0.309	1.000
124	RM243	3	120–140	0.575	0.554	0.017	0.475	0.970
125	RM467	3	200–210	0.558	0.575	0.000	0.502	1.000
126	RM564	4	250–300	0.450	0.599	0.100	0.515	0.834
127	RM8007	3	130–150	0.767	0.385	0.000	0.352	1.000
128	RM441	4	160–200	0.475	0.627	0.567	0.557	0.100
129	RM518	3	150–170	0.542	0.537	0.000	0.437	1.000
130	RM253	4	130–170	0.554	0.594	0.083	0.530	0.861
131	RM274	3	75–80	0.667	0.477	0.000	0.406	1.000
132	RM242	4	200–240	0.575	0.591	0.017	0.536	0.972
133	RM3231	4	170–550	0.346	0.703	0.650	0.645	0.080
134	RM5687	4	160–500	0.417	0.687	0.650	0.630	0.059
135	RM5626	3	165–180	0.583	0.512	0.733	0.411	−0.430
136	RM452	3	240–250	0.475	0.618	0.000	0.541	1.000
	Mean	3.74	—-	0.561	0.555	0.116	0.496	0.793

3.5. Population Genetic Structure Analysis

The diverse population for the studied antioxidant traits was genotyped for genetic structure and analyzed by adopting probable sub-populations (K) and selecting higher ΔK-value by applying the STRUCTURE 2.3.6 software. The rate of change in the log probability of data between successive K values is the delta K value used in the analysis. The panel population was categorized into two sub-populations by considering a high ΔK peak value of 362.4 at K = 2 among the assumed K (Supplementary Table S2; Supplementary Figure S2). The two subpopulations were in the proportion of 0.277 and 0.723 for population 1 and population 2, respectively. However, the subpopulations showed poor correspondence with the six antioxidant traits in the germplasm present in the studied population. Therefore, the next ΔK peak at K = 3 was compared in which the population was classified into three subpopulations. The three subpopulations showed genotypes in the proportion of 0.208, 0.689, and 0.103 in the inferred clusters for the sub-population 1, 2, and 3, respectively. The Fst1, Fst2, and Fst3 values were 0.3392, 0.1664, and 0.3701 for the sub-population 1, 2, and 3, respectively (Supplementary Table S2; Supplementary Figure S3). The ancestry value of ≥80% obtained in a genotype grouped the genotype into the particular subpopulation.

The assumed subpopulations at K = 3 differentiated the germplasm lines based on the six antioxidant traits, but did not clearly separate the SP2 and SP3 subpopulations. Hence, next ΔK peak at K = 4 was considered for the subpopulations in which the population was classified into four genetic groups. The six antioxidant traits in the studied population showed a fair degree of correspondence at K = 4 with inferred structure values in the

subpopulations. The majority of the germplasm lines with high to very high antioxidant-carrying germplasms were present in subpopulation 4. The germplasm lines showing moderate value of the antioxidant estimates are present in subpopulation 2. Germplasm lines with poor and moderate levels of antioxidant estimates were in subpopulation 1, while very poor to poor types are in subpopulation 3 (Table 3; Figure 5). The alpha value of the panel showed a low value ($\alpha = 0.0578$) estimated by the structure analysis at K = 4. Positively skewed leptokurtic distributions were observed for the mean alpha-value while normally skewed leptokurtic distributions detected for all the 4 Fst values for the panel population showing a distinct variation in the distribution among the Fst values (Supplementary Figure S4).

Table 3. The inferred ancestry value and population structure of individual member in the panel population with their antioxidant classification.

Sl. No.	Accession No./ Vernacular Name of Germplasm Line	Inferred Ancestry Value at K = 4					Antioxidants Content in Each Germplasm Line
		Q1	Q2	Q3	Q4	Group	
1	Ac. 5993	0.986	0.009	0.003	0.003	SP1	high SOD
2	Ac. 6221	0.984	0.006	0.003	0.007	SP1	Low
3	Ac. 6183	0.945	0.003	0.003	0.049	SP1	Low
4	Ac. 6170	0.994	0.002	0.002	0.002	SP1	high Carotenoid
5	Ac. 6023	0.978	0.009	0.002	0.012	SP1	high SOD
6	Ac. 6172	0.963	0.005	0.002	0.03	SP1	Low
7	Ac. 6027	0.012	0.002	0.983	0.002	SP3	Low
8	Ac. 6007	0.994	0.002	0.002	0.003	SP1	Low
9	Ac. 9006	0.973	0.006	0.009	0.012	SP1	high
10	Ac. 9021	0.927	0.053	0.005	0.015	SP1	Low
11	Ac. 9028	0.924	0.006	0.003	0.066	SP1	high GO& SOD
12	Ac. 9030	0.989	0.005	0.001	0.005	SP1	Low
13	Ac. 9035	0.959	0.021	0.017	0.003	SP1	Low
14	Ac. 9038	0.982	0.015	0.001	0.002	SP1	high SOD
15	Ac. 9043	0.95	0.046	0.002	0.002	SP1	Low
16	Ac. 9044	0.987	0.006	0.004	0.003	SP1	high SOD
17	Ac. 20920	0.51	0.48	0.007	0.004	Admix	high SOD & Carotenoid
18	Ac. 20907	0.866	0.131	0.001	0.002	SP1	high SOD
19	Ac. 20845	0.087	0.907	0.001	0.005	SP2	high Carotenoid
20	Ac. 20770	0.966	0.025	0.008	0.002	SP1	high SOD & Carotenoid
21	Ac. 20627	0.378	0.619	0.001	0.002	Admix	high Carotenoid & SOD
22	Ac. 20686	0.432	0.564	0.002	0.002	Admix	high SOD
23	Ac. 20664	0.006	0.99	0.001	0.003	SP2	Medium
24	Ac. 20614	0.109	0.887	0.003	0.001	SP2	high SOD
25	Jhagrikarti	0.97	0.02	0.002	0.008	SP1	high GO
26	Dadghani	0.963	0.03	0.003	0.004	SP1	high SOD
27	Shayam	0.004	0.002	0.993	0.002	SP3	Very low
28	Basumati	0.128	0.005	0.862	0.005	SP3	Very low
29	Bharati	0.551	0.444	0.004	0.001	Admix	high SOD
30	Joha	0.973	0.023	0.002	0.002	SP1	high SOD
31	Adira-1	0.586	0.02	0.364	0.03	Admix	Medium
32	Adira-2	0.992	0.004	0.002	0.002	SP1	Medium
33	Adira-3	0.256	0.327	0.413	0.004	Admix	Medium

Table 3. *Cont.*

Sl. No.	Accession No./ Vernacular Name of Germplasm Line	Inferred Ancestry Value at K = 4					Antioxidants Content in Each Germplasm Line
		Q1	Q2	Q3	Q4	Group	
34	PK6	0.985	0.002	0.01	0.003	SP1	Low
35	Vachaw	0.803	0.154	0.041	0.002	SP1	Medium
36	Kozhivalan	0.988	0.008	0.001	0.002	SP1	Low
37	Marathondi	0.017	0.486	0.464	0.033	Admix	Medium
38	Ezhoml-2	0.862	0.135	0.002	0.001	SP1	Medium
39	Jyothi	0.973	0.025	0.001	0.001	SP1	Medium
40	Kantakopura	0.521	0.476	0.002	0.001	Admix	Medium
41	Kantakaamal	0.055	0.585	0.207	0.153	Admix	Medium
42	Kapanthi	0.032	0.296	0.333	0.339	Admix	Low
43	Karpurkanti	0.001	0.042	0.956	0.001	SP3	Very low
44	Kathidhan	0.426	0.475	0.005	0.094	Admix	Medium
45	Kundadhan	0.005	0.992	0.001	0.002	SP2	Low
46	Champaeisia	0.005	0.991	0.002	0.002	SP2	high SOD
47	Latamahu	0.016	0.977	0.002	0.005	SP2	Medium
48	Latachaunri	0.028	0.966	0.002	0.005	SP2	high SOD
49	Ac. 10608	0.981	0.013	0.001	0.005	SP1	Low
50	Ac. 10187	0.944	0.005	0.002	0.049	SP1	Low
51	Ac. 10162	0.941	0.012	0.021	0.026	SP1	Low
52	Ac. 7282	0.003	0.002	0.995	0.001	SP3	Very low
53	Ac. 7269	0.994	0.003	0.001	0.002	SP1	Very low
54	Ac. 7134	0.749	0.032	0.21	0.009	Admix	Low
55	Ac. 7008	0.94	0.057	0.001	0.002	SP1	Low
56	Ac. 9093	0.99	0.005	0.004	0.001	SP1	high SOD
57	Ac. 9090	0.958	0.022	0.016	0.004	SP1	high SOD
58	Ac. 9076A	0.844	0.148	0.001	0.007	SP1	Low
59	Ac. 9065	0.923	0.012	0.061	0.004	SP1	Low
60	Ac. 9063	0.667	0.324	0.001	0.008	Admix	GO & SOD
61	Ac. 9058	0.992	0.005	0.001	0.001	SP1	Low
62	Ac. 9053A	0.852	0.007	0.014	0.127	SP1	Low
63	Ac. 9050	0.894	0.097	0.007	0.002	SP1	Low
64	Ac. 9005	0.985	0.009	0.003	0.004	SP1	high SOD
65	Ac. 20389	0.963	0.004	0.008	0.026	SP1	high Carotenoid & SOD
66	Ac. 20371	0.976	0.019	0.001	0.004	SP1	high GO & SOD
67	Ac. 20423	0.975	0.019	0.001	0.005	SP1	Medium
68	Ac. 20362	0.968	0.013	0.006	0.013	SP1	high SOD
69	Ac. 20328	0.804	0.172	0.014	0.009	SP1	high SOD
70	Ac. 20317	0.882	0.089	0.027	0.003	SP1	high SOD
71	Ac. 20282	0.536	0.339	0.009	0.116	Admix	high GO & SOD
72	Ac. 20246	0.639	0.262	0.069	0.03	Admix	high SOD & Carotenoid
73	Ac. 20347	0.927	0.029	0.002	0.042	SP1	high SOD & Carotenoid
74	Palinadhan-	0.321	0.038	0.381	0.26	Admix	high SOD
75	Chatuimuchi	0.001	0.001	0.996	0.001	SP3	high SOD
76	Uttarbangal	0.743	0.155	0.002	0.101	Admix	high SOD
77	Gochi	0.943	0.007	0.007	0.043	SP1	high SOD

Table 3. *Cont.*

Sl. No.	Accession No./ Vernacular Name of Germplasm Line	Inferred Ancestry Value at K = 4					Antioxidants Content in Each Germplasm Line
		Q1	Q2	Q3	Q4	Group	
78	Sugandha-2	0.003	0.002	0.995	0.001	SP3	high SOD
79	Jhingesal	0.365	0.631	0.001	0.002	Admix	high SOD
80	Cheruviripp	0.852	0.142	0.002	0.004	SP1	Low
81	Mahamaga	0.548	0.399	0.002	0.051	Admix	Very low
82	Jaya	0.928	0.064	0.001	0.007	SP1	Low
83	D1	0.89	0.042	0.019	0.049	SP1	Low
84	PK21	0.705	0.27	0.002	0.023	Admix	Low
85	Gandhakasal	0.002	0.086	0.908	0.004	SP3	high SOD
86	Sreyas	0.909	0.085	0.003	0.002	SP1	Medium
87	Gondiachampeisiali	0.011	0.986	0.002	0.002	SP2	high SOD
88	Chinamal	0.229	0.761	0.008	0.002	Admix	high SOD
89	Magra	0.267	0.726	0.005	0.003	Admix	high SOD
90	Landi	0.011	0.986	0.002	0.002	SP2	Low
91	Lalgundi	0.005	0.988	0.004	0.003	SP2	high SOD
92	Balisaralak	0.004	0.99	0.002	0.003	SP2	VL, L, SOD
93	Laxmibilash	0.005	0.465	0.527	0.003	Admix	Very low
94	Kaniar	0.03	0.958	0.006	0.007	SP2	high Carotenoid & SOD
95	Kanakchampa	0.037	0.95	0.009	0.004	SP2	high SOD
96	Magura-S	0.003	0.984	0.012	0.001	SP2	high SOD
97	Ac. 44603	0.014	0.017	0.001	0.967	SP4	high Carotenoid & SOD
98	Ac. 44585	0.005	0.003	0.012	0.981	SP4	Low
99	Ac. 44598	0.02	0.003	0.01	0.968	SP4	high Carotenoid
100	Ac. 44592	0.001	0.001	0.014	0.984	SP4	high Carotenoid, TFC, ABTS
101	Ac. 44646	0.002	0.001	0.001	0.996	SP4	High Carotenoid, TAC, TFC, SOD, ABTS
102	Ac. 44604	0.028	0.004	0.012	0.956	SP4	high Carotenoid & SOD
103	Ac. 44597	0.002	0.003	0.001	0.994	SP4	high TFC & Carotenoid
104	Ac. 44638	0.001	0.001	0.701	0.297	Admix	Low
105	Ac. 44595	0.007	0.003	0.011	0.978	SP4	high SOD, Carotenoid, ABTS
106	Ac. 44588	0.002	0.001	0.001	0.995	SP4	High ABTS
107	Ac. 44591	0.002	0.002	0.001	0.995	SP4	high Carotenoid & SOD
108	Ac. 44594	0.011	0.006	0.002	0.981	SP4	high SOD
109	Ac. 43737	0.003	0.002	0.002	0.993	SP4	high TAC & SOD
110	Ac. 43660	0.003	0.003	0.001	0.993	SP4	high Caro, TAC, TFC, SOD, ABTS
111	Ac. 43732	0.002	0.001	0.001	0.995	SP4	high SOD & ABTS
112	Ac. 43661	0.006	0.004	0.001	0.989	SP4	high SOD
113	Ac. 43738	0.002	0.004	0.002	0.992	SP4	high SOD, ABTS, TAC
114	Ac. 43669	0.006	0.004	0.003	0.987	SP4	high Caro, TAC, TFC, SOD
115	Ac. 43663	0.002	0.002	0.002	0.994	SP4	high SOD
116	Ac. 43658	0.001	0.001	0.001	0.997	SP4	High TAC & SOD
117	Ac. 43662	0.004	0.002	0.027	0.967	SP4	High SOD
118	Ac. 43670	0.003	0.003	0.18	0.815	SP4	High SOD, ABTS, TAC
119	Ac. 43675	0.003	0.002	0.014	0.98	SP4	High TAC, SOD
120	Ac. 43676	0.007	0.015	0.043	0.935	SP4	High SOD

3.6. Molecular Variance (AMOVA) and LD Decay Plot Analysis

The closely related plants among themselves in a population are grouped into isolated subpopulations. The genetic variations obtained within and between the subpopulations at K = 4 were estimated by the analysis of molecular variance (AMOVA) (Table 4). The genetic variations estimated at K = 4 was computed to be 6% among the populations, nil among individuals, and there was 94% variation within individuals of the panel population. Wright's F statistics was used to obtain the deviation from the Hardy–Weinberg prediction. The parameter F_{IS} was used to analyze the uniformity of individuals within the subpopulation and F_{IT} for individuals within the total population for differentiation of the population. The F_{IT} and F_{IS} of the total population and within population estimated on the basis of 136 marker loci showed −0.148 and 0.235, whereas the total population had a F_{ST} value of 0.071 between the four subpopulations. Fst is used to identify the subpopulations or population differentiation within the total population. A clear differentiation among the four subpopulations was observed for the Fst values from each other in their distribution pattern (Supplementary Figure S4).

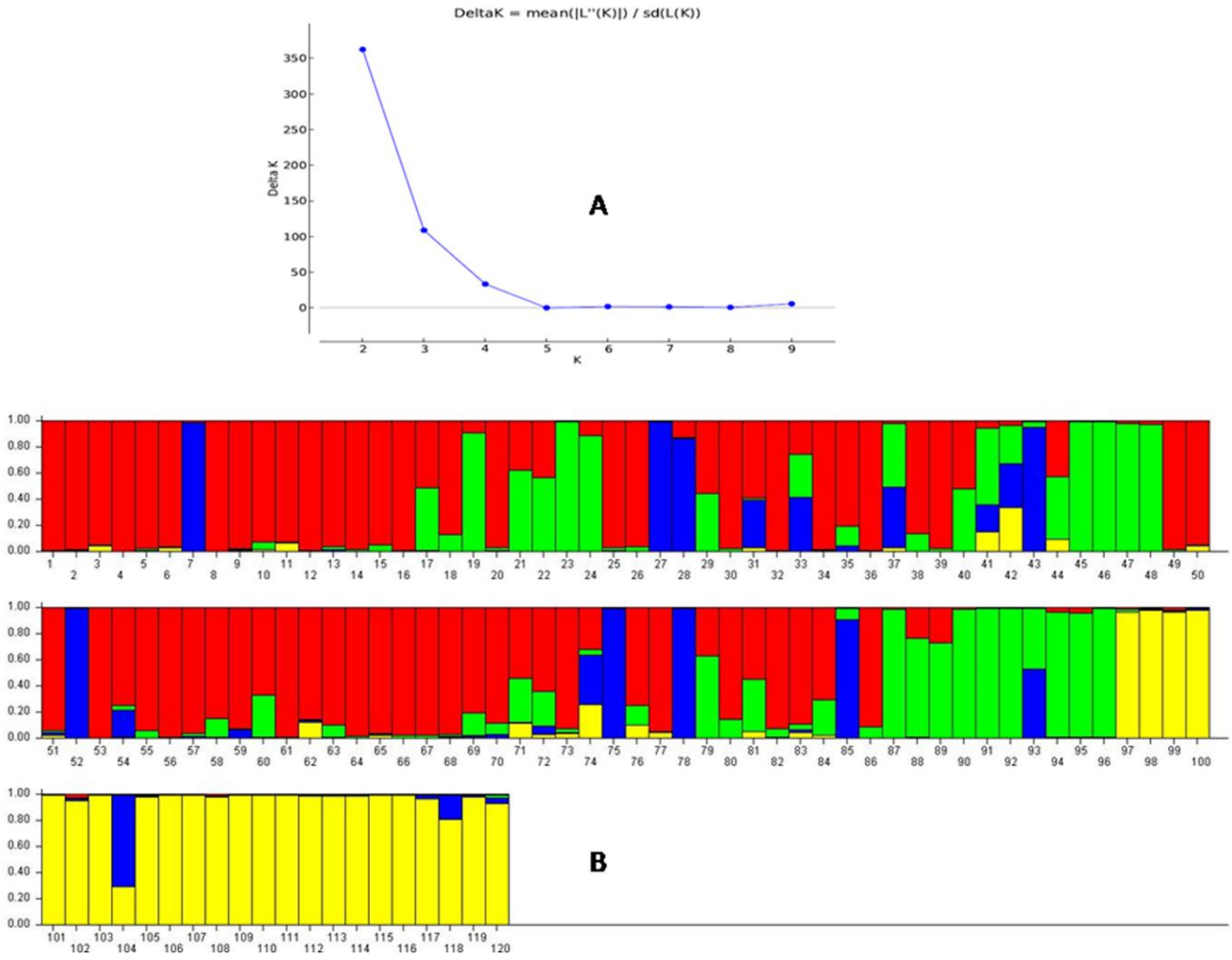

Figure 5. (**A**) Graph of ΔK value, to the rate of change in the log probability of data between successive K values. (**B**) Population structure of the panel population based on membership probability fractions of individual genotypes at K = 4. The genotypes with the probability of ≥80% membership proportions were assigned as subgroups, while others were grouped as admixture group. The numbers in the diagram depict the serial number of the germplasm lines listed in the Table 1.

Table 4. Analysis of molecular variance (AMOVA) of the sub-populations of the panel population for the antioxidant traits in the 120 rice genotypes.

Source of Variation	AMOVA for the Four Subpopulations at K = 4			
	Df.	**Mean Sum of Squares**	**Variance Components**	**Percentage Variation**
Among populations	4	551.634	2.575	6%
Among individuals (accessions) within population	115	2983.721	0.000	0%
Within individuals (accessions)	120	5027.000	41.892	94%
Total	239	8562.354	44.467	100%
F-Statistics	Value		*p*-Value	
Fst	0.071	0.001		
F_{IS}	−0.235	1.000		
F_{IT}	−0.148	1.000		
F_{ST} max.	0.501			
F'_{ST}	0.141			

The association of alleles by different loci in a nonrandom manner is utilized in the marker–trait association analysis. Existence of marker–trait association is dependent on the LD decay rate in a population over a time period. The LD decay rate will indicate the possibility of new genes or allelic variants controlling the antioxidant compounds associated with molecular markers for these traits. A syntenic r^2 value was used to plot the linkage disequilibrium decay of the population versus the physical distance in million base pair (Figure 6A). Tightly linked markers had higher r^2 values and the average r^2 values rapidly decreases for increase in linkage distance. In the LD plot, it is observed that the LD decay in the beginning was delayed in the studied panel populations. However, a decline of LD decay was noticed in the curve for the associated markers at about 1–2 mega base pair and there, after a gradual and very slow decay, this can be noticed in the graph. The graph clearly indicates the continuance of linkage disequilibrium decay in the population for the studied antioxidant traits in the population. The limitation for LD decay depends on non-random mating, mutation, selection, migration or admixture, and genetic drift, which will influence the estimates of LD. This LD decay plot also provides a clue for the creation of genetic admixture groups for various antioxidants traits in the population. A similar trend was also noticed in the marker 'P' versus the marker 'F' and marker R^2 (Figure 6B) curve. The detected markers from this study indicated the strength of the markers for the studied antioxidant compounds.

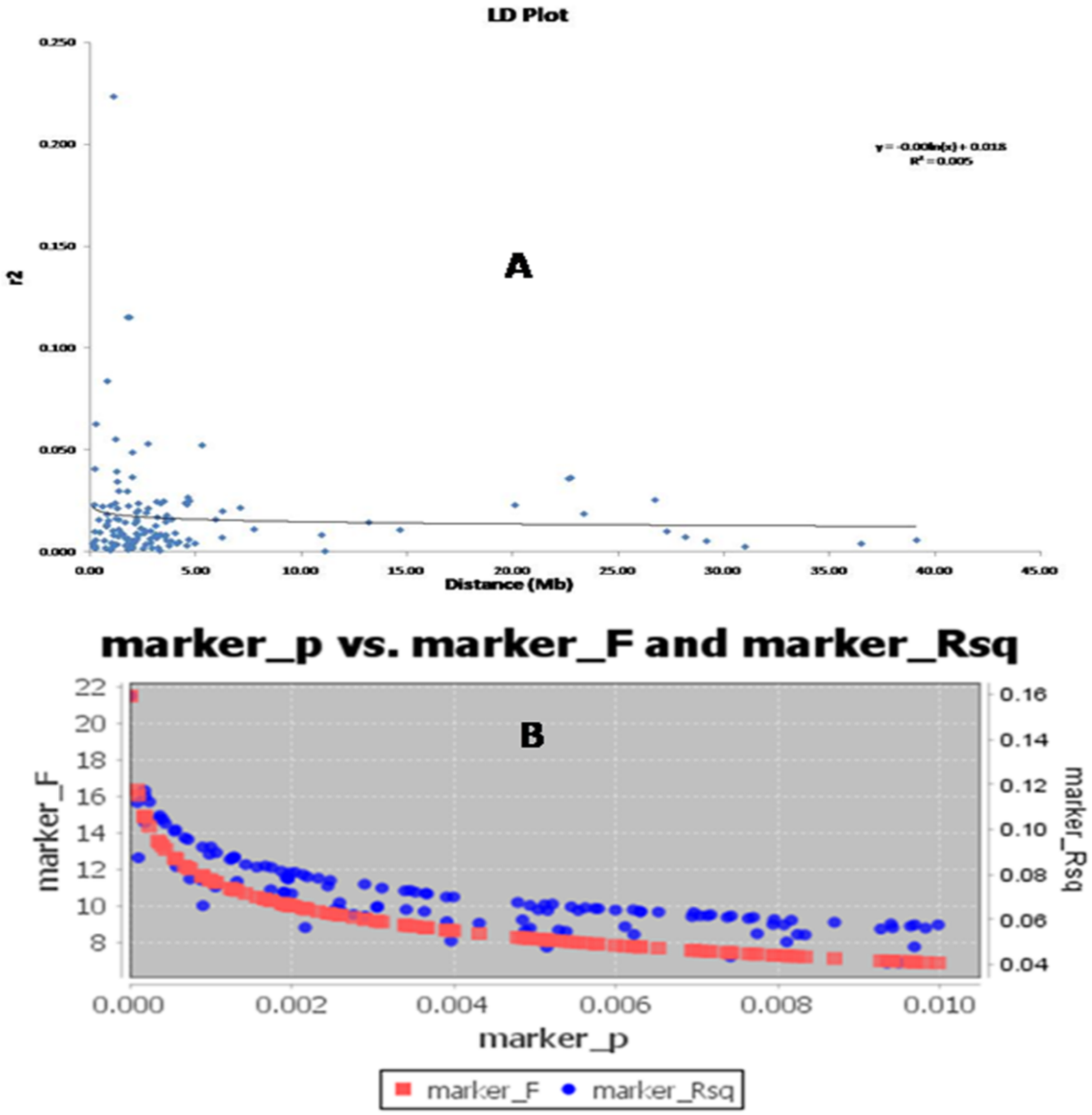

Figure 6. (**A**) The physical distance (base pairs, bp) between pairs of loci on chromosomes against linkage disequilibrium (LD) decay (r^2) curve plotted in rice. The decay started in million bp estimated by taking the 95th percentile of the distribution of r^{2R2} for all unlinked loci. (**B**) The marker 'P' versus marker 'F' and marker R^2.

3.7. Principal Coordinates and Cluster Analyses for Genetic Relatedness among the Germplasm Lines

The two-dimensional plot for the principal coordinate analysis (PCoA) was constructed based on the genotyping data of 136 SSR markers which classified the 120 germplasm lines as per the genetic relatedness among the lines (Figure 7). The inertia showed by component 1 was 11.73%, while 7.49% exhibited by component 2. The germplasm lines were allotted different spots in the four quadrants forming 3 major groups (Figure 7). The biggest group accommodated all the germplasm lines of the subpopulation 2 and 3 together and clustered in the 2nd (bottom right) quadrant. The genotypes in the 1stquadrant are divided into 2 groups, of which one group on the top of the 1st quadrant forms the SP3 subpopulation which showed mostly low to very low estimates for the antioxidant traits in the seeds. The other group near to the axis1 is for all the admix types of the germplasm lines. Few germplasm lines of quadrant II and closer to the axis 1 are also admix genotypes. Then, admix genotypes present on both sides of axis 1 are depicted in black color (Figure 7).

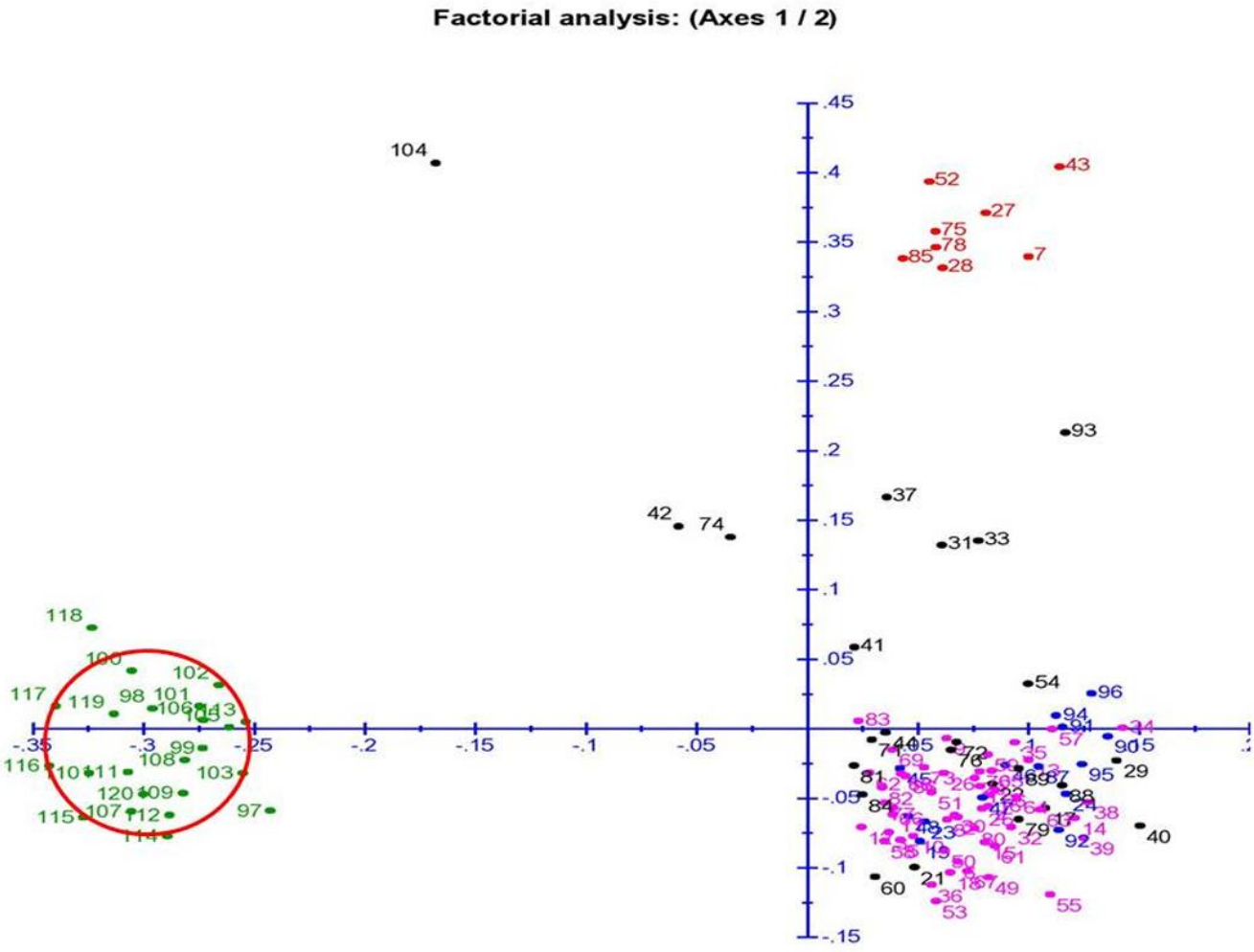

Figure 7. Principal coordinate analysis (PCoA) of 120 genotypes in the panel population for the 6 antioxidant traits using 136 molecular markers. The dot numbers in the figure represent the serial number of the genotypes enlisted in the Table 1. The numbers are colored on the basis of sub-populations obtained from structure analysis (SP1: Pink; SP2; Blue; SP3: Red; SP4: Green, and Admix: Black).

The germplasm lines containing high to very high estimates of antioxidant traits are grouped together, forming subpopulation 4. This subpopulation is present on quadrant III (top left) and IV (bottom left) and encircled in red color. The germplasm lines rich in antioxidants are placed on both sides of the axis 1 on the quadrant III and IV (Figure 7). The PCoA distributed all the germplasm lines into the four quadrants classifying them into 4 clusters and a separate admixture group. The subpopulations clustered by PCoA showed correspondence with the population structure (Figure 7). Germplasm lines namely Ac. 44594, Ac. 43669, Ac. 44597, Ac. 44588, Ac. 43737, Ac. 44595, Ac. 43676, Ac. 44597, Ac. 44592, Ac. 43738, and Ac. 44646 are placed together in one structure group present in quadrants III and IV and are rich in antioxidants. The PCoA placed germplasm lines in quadrant II which were mostly average in the antioxidant traits. This quadrant formed the group by placing all the germplasm lines of subpopulations 1 and 2.

As per the Ward clustering, all the germplasm lines were broadly grouped into two major groups. The largest cluster, cluster 1, accommodated 111 germplasm lines in which most of the lines showed poor to average for the antioxidant estimates. The cluster II had nine germplasm lines only. The dendrogram placed all the germplasm lines in this cluster II which were rich for the antioxidant traits. This cluster again subdivided into

2 subgroups, which were further divided into six sub-subclusters. Cluster I was divided into two main sub clusters which finally divided into 32 small groups. All the clusters and small groups accommodated in the Ward clustering approach were based on the antioxidant traits estimates in the germplasm lines (Figure 8A). The cluster analysis discriminated the germplasm lines on the basis of markers data of 136 SSR markers and placed the genotypes into different clusters which corresponded with the studied antioxidant level in the germplasm. The unweighted-neighbor joining tree differentiated the genotypes into four different clusters (Figure 8B). The cluster for subpopulation 4 was differentiated from SP_2 by the presence of germplasm lines containing high antioxidants in it, while moderate to high-containing genotypes were in subpopulation 2. The green-colored portion of the tree is designated as SP4 while blue for SP2. The very poor in antioxidant traits in the germplasm lines were in the subpopulation 3 those depicted in red color in the tree. The majority of the germplasm lines present in subpopulation 1 were poor to medium in antioxidant value and are shown in pink color. The germplasm lines with admix type of population are depicted in black color in the neighbor joining tree (Figure 8B).

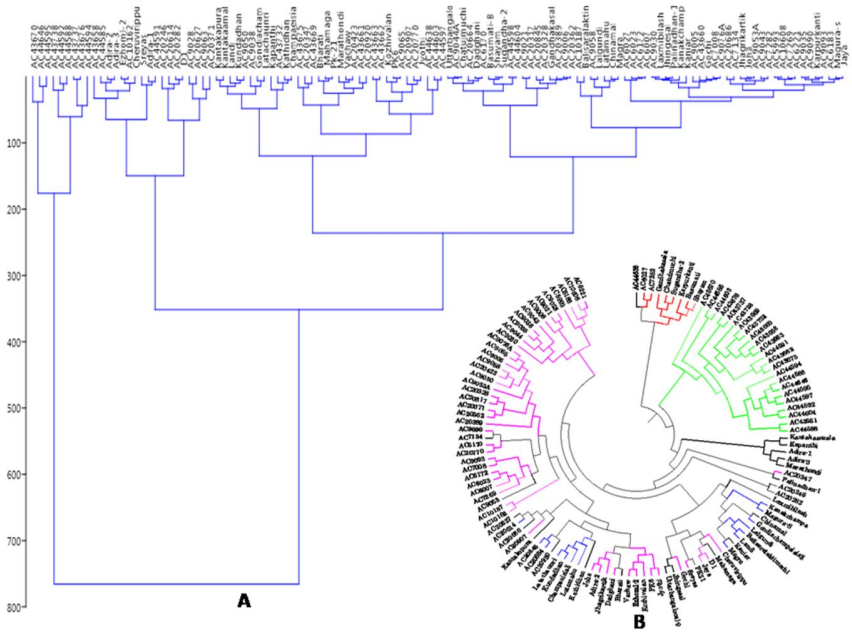

Figure 8. (**A**) Grouping of the panel germplasm lines A. Ward's clustering based on the antioxidant content (**B**) Unrooted tree using unweighted-neighbor joining method depicting clustering pattern of 120 germplasm lines with respect to 136 molecular markers colored on the basis of subpopulations obtained from structure analysis (SP1: Pink; SP2; Blue; SP3: Red; SP4: Green, and Admix: Black).

3.8. Marker–Trait Association for Antioxidant Traits in the Rice Panel Population

Marker–trait associations were computed for the six antioxidant traits by using Generalized Linear Model (GLM) and Mixed Linear Model (MLM/K + Q model)) in the TASSEL 5 software. The marker–trait association values were compared at less than 1% error i.e., 99% confidence ($p < 0.01$). A total of 57 and 23 significant marker–trait associations were detected for five antioxidant traits by GLM and MLM, respectively, at $p < 0.01$. The range for marker R^2 values was from 0.0477 to 0.159 by GLM while 0.0607 to 0.1169 detected by Mixed Linear Model (Supplementary Table S3; Supplementary Table S4). A total of 14 significant marker–trait associations were detected by both the models for five antioxidant traits present in the seed at $p < 0.01$ (Figure 9A). Significant association of 5 SSR markers with TAC; 3 with SOD, TFC; 2 with GO, and ABTS were detected. Five antioxidant compounds present in the studied germplasm lines presented a higher marker R^2 (>0.1) with low *p*-values (<0.01) in the associations study includes SOD with RM405 and GO with

RM3701 (Table 5; Figure 9A). The Q-Q plot also confirmed the association of these markers with the associated antioxidant traits in rice (Figure 9B).

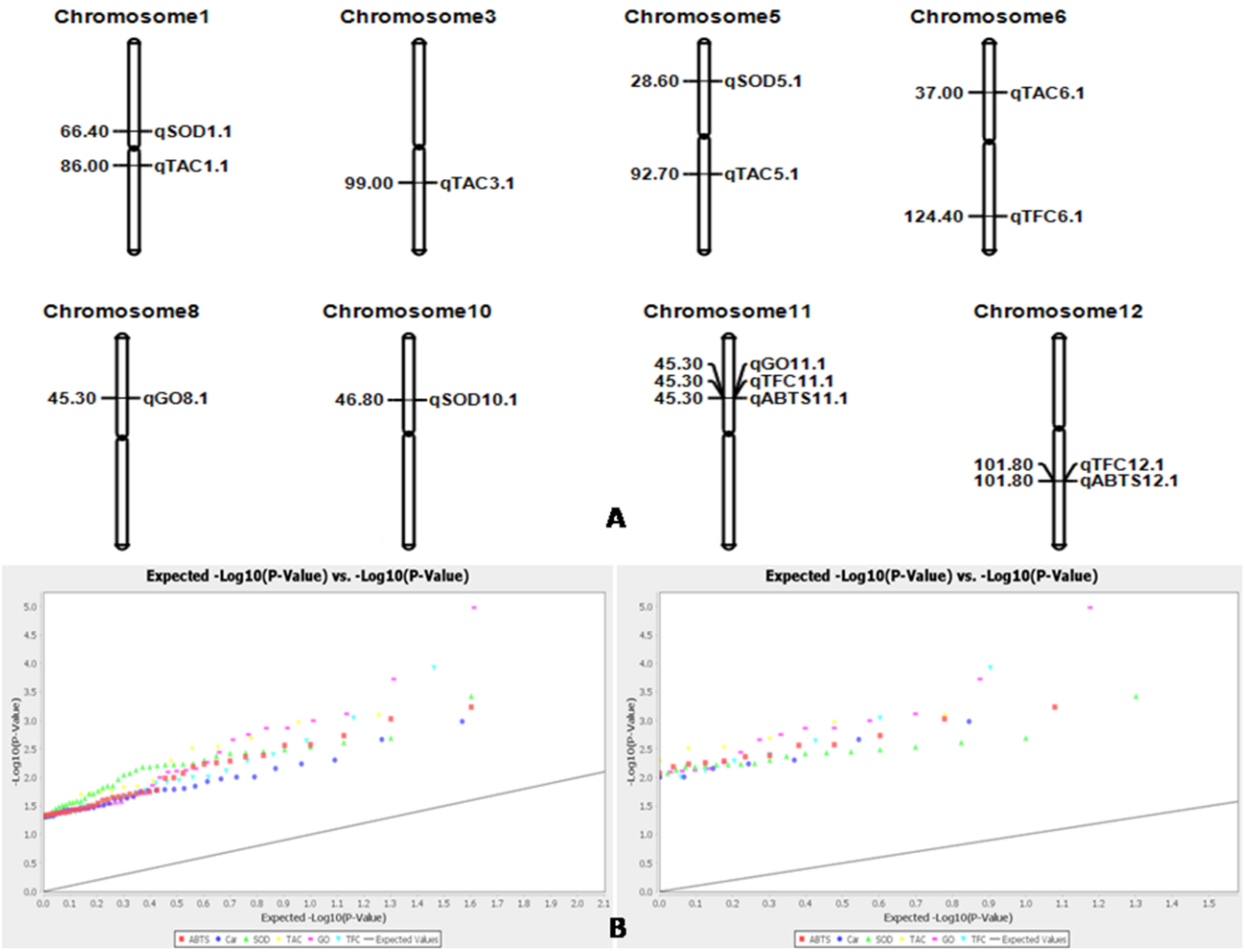

Figure 9. (**A**) Positions of the QTLs on the chromosomes for antioxidant content detected by association mapping in rice. (**B**) Distribution of marker–trait association and quantile–quantile (Q-Q) plot generated from Generalized Linear Model analysis for six antioxidant traits at (**A**) $p < 0.05$ and (**B**) at $p < 0.01$.

Table 5. Marker–trait associations with antioxidant content in the panel population detected by both the models of GLM and MLM at $p < 0.01$.

Sl. No	Antioxidant Compounds	Marker	Position (cM)	GLM				MLM				
				Marker_F	Marker_p	Marker_R^2	q-Value	Marker	Marker_F	Marker_p	Marker_R^2	q-Value
1	SOD	RM582	66.4–66.4 cM	7.51326	0.00713	0.0617	0.0617	RM582	10.35724	0.00169	0.09191	0.005571
2	SOD	RM405	28.6–28.6 cM	8.28345	0.00479	0.06759	0.06759	RM405	12.0128	7.52×10^{-4}	0.10661	0.005571
3	SOD	RM467	46.8–46.8 cM	9.70831	0.00233	0.07829	0.07829	RM467	9.70377	0.00234	0.08612	0.005571
5	TAC	RM440	92.7–92.7 cM	10.07764	0.00194	0.06646	0.06646	RM440	9.06064	0.00323	0.08013	0.005726
6	TAC	RM5638	86–86 cM	12.02036	7.47×10^{-4}	0.07803	0.07803	RM5638	11.04573	0.0012	0.09768	0.005571
7	TAC	RM253	37–37 cM	11.30677	0.00106	0.07443	0.07443	RM253	10.51261	0.00157	0.09297	0.005571
8	TAC	RM5626	99–99 cM	9.36875	0.00276	0.06215	0.06215	RM5626	9.35822	0.00278	0.08276	0.005571
9	GO	RM3701	45.3–45.3 cM	14.94433	1.87×10^{-4}	0.11729	0.11729	RM3701	9.33336	0.00282	0.08155	0.005571
10	GO	RM502	121.8–121.8 cM	21.52493	9.54×10^{-6}	0.15935	0.15935	RM502	8.35407	0.00463	0.073	0.006936
11	TFC	RM3701	45.3–45.3 cM	11.62841	9.06×10^{-4}	0.06613	0.06613	RM3701	8.95629	0.00341	0.07279	0.005782
12	TFC	RM235	101.8–103.8 cM	16.06018	1.11×10^{-4}	0.08746	0.08746	RM235	9.20885	0.003	0.07484	0.005571
13	TFC	RM494	124.4–124.4 cM	9.85164	0.00217	0.05638	0.05638	RM494	9.64481	0.00241	0.07839	0.005571
14	ABTS	RM3701	45.3–45.3 cM	12.55463	5.79×10^{-4}	0.08346	0.08346	RM3701	10.97479	0.00125	0.09699	0.005571
15	ABTS	RM235	101.8–103.8 cM	8.08868	0.0053	0.05533	0.05533	RM235	7.06457	0.00902	0.06243	0.009257

Four markers, namely, RM440, RM5638, RM253, and RM5626, showed significant associations with compound, TAC detected by GLM and MLM models at $p < 0.01$, showing >0.05 marker R^2 value. The QTLs controlling anthocyanin content in these genotypes are detected to be located near the markers present at RM440, RM5638, RM253, and RM5626 at 92.7, 86, 37, and 99 cM on the chromosome 5, 1, 6, and 3, respectively. Three markers, namely, RM582, RM467, and RM405, located at 66.4, 46.8, and 28.6 cM positions on chromosome 1, 10, and 5, respectively, were associated with the compound SOD. TFC content was detected to be associated with markers RM 3701, RM235, and RM494 present at 45.3, 101.8, and 124.4 cM on chromosome 1, 11, and 12, respectively. The QTLs for ABTS activity showed significant associations with RM3701 and RM235 on chromosomes 1 and 11, respectively. The marker RM216 showed association with SOD at very low *p*-value and high marker R^2 value of >0.10618 analyzed by the GLM only. The QTLs for antioxidant compound, OZ, showed significant associations with RM3701 and RM502 on chromosomes 1 and 8, respectively (Table 5; Figure 9A). The Q-Q plot also confirmed the associations of these markers with the estimated antioxidant compounds in rice (Figure 9).

Association mapping studies for the antioxidant traits in seeds identified co-localization of QTLs controlling the antioxidant traits in rice. It is observed that the same marker showed significant associations with different antioxidant traits in rice by both models (Table 5). Significant associations of marker RM3701 with the antioxidant traits GO, TFC, and ABTS estimated from the germplasm lines were detected. In addition, it was also detected the association of RM235 with the traits TAC, TFC, and ABTS by both the models at <1% error and $p < 0.01$ (Table 5). While considering the marker association analyzed by GLM, the marker RM494 showed association with both carotenoids and TFC. In addition, RM494 was associated with both the traits, SOD and TFC analyzed by the model, MLM.

4. Discussion

The genotypes shortlisted for the six antioxidant traits mapping exhibited wide genetic variation among themselves (Supplementary Table S1; Table 1). In addition, significant correlation was observed between few antioxidant traits *viz*., TAC with TFC, TFC with ABTS, and TAC with ABTS. Existence of genetic variation and correlation for these traits provide enough insight about the possibility for improvement of the antioxidant traits in rice (Tables 1 and 4). Earlier reports of high variations for antioxidant traits were also published by few researchers [17,35,45,66–68]. The available diversity in the population based on 136 markers data for the six antioxidant traits represented clear-cut groups in the studied population (Table 2). A moderate to high PIC value coupled with better informative markers in the studied population will be useful for improvement of the antioxidant breeding program. The Jeypore tract of Odisha is known for being a secondary center of origin of rice, and germplasms from this tract were also included in this study. Additionally, the shortlisted germplasm lines used as materials in this study were collected from states known for their rich rice genetic diversity [35,45,69]. The genotypes rich in multiple antioxidant traits were estimated from the germplasm lines Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669. These germplasm lines will be good source materials in the antioxidant improvement programs (Table 1; Supplementary Table S2). Therefore, it is expected that the breeding program with inclusion of parental lines from this population will be effective in terms of antioxidants' improvement in rice. The assumed subpopulations at K = 3 differentiated the members different subpopulations for the 6 antioxidant traits but did not clearly separate the SP2 and SP3 subpopulations. Therefore, the next ΔK peak at K = 4 was considered for the subpopulations in which the population was classified into four genetic groups. The six antioxidant traits in the studied population showed a fair degree of correspondence at K = 4 with inferred structure values in the subpopulations. Structure analysis categorized the population into four subpopulations (K = 4), showing different Fst values, supporting the availability of the linkage disequilibrium groups in the population. The detection of a low alpha value and the existence of many genetic admix-type germplasm lines in the population indicated that the antioxidant traits evolved from a

single source initially during evolution of the trait. Different antioxidant compounds were subsequently formed by admix genotypes with different ancestry value during evolutionary process. A similar view of the evolution of complex traits was reported by earlier publications based on the admix genotypes [5,8,9,70]. Population genetic structure group and its correspondence with the traits in each group are important for obtaining a marker–trait association. A good correspondence of genetic structure and different traits was previously published by many researchers [36,61,71]. Additionally, publications on the phenotype of various traits and structure correlation have been published by many workers [45,46,66,67,72].

Five antioxidant compounds were found to be associated with 12 SSR markers analyzed by both GLM and MLM approaches (Table 5). The markers' association detected by both the models at $p < 0.01$ and low p-value are considered to be very robust and useful markers for improvement program. The strongly associated SSR markers, namely, RM440, RM235, RM5638, RM253 and RM5626 for TAC; RM582 and RM467 for SOD; RM 3701, RM235 and RM494 for TFC; RM3701 and RM235 for ABTS; RM3701 and RM502 for GO, will be useful markers for selection of antioxidant carrying plants (Table 5). The Q-Q plot also confirmed the associations of these markers with the antioxidant compounds in rice (Figure 9B).

The QTLs for anthocyanin and proanthocyanin content in rice were reported by earlier researchers [19,23,30]. In the present investigation, the QTLs for total anthocyanin content were detected on chromosomes 1, 3, 5, and 12. The QTLs on chromosomes 1 and 3 were at position of 86 cM and 99 cM, respectively. The genes *qANC3* and *qPAC12-2* reported by Xu et al. [19] were at the same position as in the present investigation. Therefore, these two QTLs were validated in our study using the present mapping population. However, another two QTLs located on chromosome 1 and 5 detected in this investigation were not reported by earlier researchers. These two QTLs may be new loci which affect TAC in rice and are designated as *qTAC1.1* and *qTAC5.1*. Three markers, namely, RM582, RM405, and RM467, showed an association with SOD and were located on chromosomes 1, 5 and 10 at 66.4, 28.6, and 46.8 cM, respectively. The QTLs reported by [23,27] for anthocyanin content in rice were at different position than the locations detected by us on chromosomes 1,3, and 6. Saini et al. [73] reported 23 QTLs located on chromosome 3,5,6,7, and 9. We detected QTLs for the trait on chromosome 1, 5, and 10. The detected QTLs by Saini et al. [73] on chromosome 5 were quite away from the QTLs detected by us. In addition, no report of QTL from the earlier studies on chromosome 1 and 10 which were detected by us at 15.34 Mb and 13.48 Mb positions, respectively. Therefore, all the 3 QTLs were not reported in earlier studies. These QTLs designated as *qSOD1.1*, *qSOD5.1*, and *qSOD10.1* may be new loci controlling the SOD activities in rice seeds. The total flavonoids content (TFC) is detected to be associated with three regions on chromosomes 6, 11, and 12. The earlier publication of Shao et al. [10] showed the presence of QTLs on chromosome 4, 7, 8, 9, and 10 [10]. The main flavonoids structural genes located on chromosome 11 for CHS [74]; on chromosome 3 for CHI [36]; on chromosome 4 for F3H [75]; on Chromosome 1 for DFR [75] and ANS [72]. The gene CHS on chromosome 11 was at 3.3 cM. We detected it at 45.3 cM. Therefore, all these three detected QTLs which affect total flavonoids are new loci and are designated as *qTFC6.1*, *qTFC11.1*, and *qTFC12.1*. Zhang [76] reported four QTLs controlling flavonoid content in rice grain located on chromosome 4. However, we detected three QTLs on chromosomes 6, 11, and 12. Therefore, the detected QTLs by us regulating flavonoid content in rice were not reported in earlier studies.

Food containing γ-oryzanol (OZ) is well recognized for its health benefits. This is a mixture of several compounds present in the rice bran layer. The γ-oryzanol content in this study showed significant association with markers on the chromosomes 8 and 11. However, QTLs previously reported by earlier workers reported on the chromosome 1, 5, and 9 in Asominori/IR24 RILs [34]. However, they detected another 5 QTLs for OZ in the backcross lines of Sasanishiki/Habataki/Sasanishiki. These two new loci detected in this investigation are new loci controlling γ-oryzanol, and are designated as *qOZ8.1* and *qOZ11.1*. The

QTLs for ABTS activities showed significant associations with RM3701 and RM235 on chromosomes 11 and 12, respectively. The candidate gene controlling ABTS and present on the chromosome 11 is not reported by earlier researchers. Hence, the detected QTL for ABTS on chromosome 11 at 45.3 cM position is a new locus controlling the trait, and it is designated as *qAC11.1*. However, the other detected association for the trait on chromosome 12 is located in the 26.1 Mb position. An earlier mapping publication reported the gene on the chromosome 12 at 25.2 Mp position [33]. As our detected QTL position for ABTS activity is close to the reported QTL *qAC12*, this QTL is validated in our mapping population and can be useful in the marker-assisted breeding for ABTS improvement.

Two markers were observed to be associated with more than one antioxidant trait analyzed by both the models at <1% error and $p < 0.01$. Marker RM3701 showed associations with antioxidant traits, GO, TFC, and ABTS present in the germplasm lines. Additionally, RM235 was associated with traits, TAC, TFC, and ABTS by both models (Table 5). These observations indicated the close location of the candidate genes and simultaneous inheritance of these QTLs are expected in the progenies. Hence, simultaneous improvement of both these antioxidant traits will be effective. These genomic locations are considered as chromosome hot spots and are very useful in improvement programs. Recent publications have also suggested easy improvement of the co-localized genes controlling various traits in rice [6,45,76]. Results of the present investigation showed that association mapping is an effective method to detect potential loci for antioxidant traits in rice. The detected loci will further be fine-mapped for application in maker-assisted breeding for improvement of antioxidant traits in rice.

5. Conclusions

Consumption of rice containing a higher content of antioxidants has many health benefits. Donor lines rich in more than three antioxidant traits were identified from the population. The germplasm lines, namely, Ac. 44592, Ac. 44646, Ac. 44595, Ac. 43660, Ac. 43738, Ac. 43660, and Ac. 43669, presented high results for three antioxidant traits. Antioxidant traits such as superoxide dismutase, flavonoids, anthocyanins, γ-oryzanol, and ABTS were mapped in a representative panel population using 136 SSR markers through association mapping. Wide genetic variations were observed for the studied six antioxidant traits in the population. The population was classified into four genetic structure groups by the structure analysis. The existence of linkage disequilibrium for the antioxidant traits was established based on the population's fixation indices. The population was classified into four subpopulations which showed a fair degree of correspondence with the antioxidant traits present in each subpopulation. A total of 14 significant marker–trait associations for the antioxidant traits were detected of which 3 QTLs namely *qANC3*, *qPAC12-2* for anthocyanin content and *qAC12* for ABTS activity were validated in the population. These three QTLs are useful in the marker-assisted breeding programs. Eleven putative QTLs, such as *qTAC1.1* and *qTAC5.1* for anthocyanin content; *qSOD1.1*, *qSOD5.1* and *qSOD10.1* for SOD; *qTFC6.1*, *qTFC11.1*, and *qTFC12.1* for TFC; *qOZ8.1* and *qOZ11.1* for γ-oryzanol, and *qAC11.1* for ABTS, were detected as novel loci. Co-localization of the QTLs detected for *OZ11.1*, *TFC11.1*, and *AC11.1* regulating γ-oryzanol, flavonoid, and anthocyanin content, respectively, while *PAC12.2* for anthocyanin content remained closer to *TFC12.1* for flavonoid content. These strongly associated QTLs will be useful in the antioxidant improvement programs in rice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123036/s1, Figure S1: Scree plot and loadings generated by the six antioxidant traits and eigen values % in the 120 rice germplasm lines. Figure S2: (A) Graph of ΔK value, to the rate of change in the log probability structure of the 120 germplasm lines of the panel population based on membership probability fractions of individual genotypes at K of data between successive K values; (B) Population = 2. Figure S3: (A) Graph of ΔK value, to the rate of chan structure of the 120 germplasm lines of the panel population based on membership probability fractions ge in the log probabil-

ity of data between successive K values; of individual genotypes at K (B) Population = 3. Figure S4: The distribbuuti pattern of alpha value and Fst values in the 4 subpopulations at K = 4. Table S1: Mean vaalues of carotenoids, SOD, TAC, GO, TFC and AABTS antioxidants in 270 rice germplaassm line. Table S2: The inferred ancestry value and population structure of individual member with their antioxidants classification in the panel population at K = 2 & K = 3. Table S3: Marker-trait associations with antioxidant content in the panel population detected by the model GLM at $p < 0.01$. Table S4: Marker-trait associations with antioxidant content in the panel population detected by the model MLM at $p < 0.01$. Table S5: Markers information of the selected 136 SSR markers used for antioxidant content in indica rice.

Author Contributions: S.K.P. conceived the study; R.B., E.P., S.R.B., D.K.N., A.S., A.M. and B.K.J. performed the genotyping work; P.S., J.M., N.B., K.C.P., R.R., S.L. and S.K.P. performed the phenotyping work; and P.K.D. and D.L. analyzed the data; S.K.P. and P.K.D. wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable as the study did not involve humans.

Informed Consent Statement: Not applicable as the study did not involve humans.

Data Availability Statement: The data generated or analyzed in this study are included in this article.

Acknowledgments: The authors are highly grateful to the Head, Crop Improvement Division and Director, ICAR-NRRI, Cuttack for encouraging the team and providing all the necessary facilities.

Conflicts of Interest: The authors declare that there is no competing interest and that the article is submitted without any commercial or economic interest that could be generated as a potential conflict of interest.

References

1. Shridhar, G.; Rajendra, N.; Murigendra, H.; Shridevi, P.; Prasad, M.; Mujeeb, M.A.; Arun, S.; Neeraj, D.; Vikas, S.; Suneel, D.; et al. Modern Diet and its Impact on Human Health. *J. Nutr. Food Sci.* **2015**, *5*, 6. [CrossRef]
2. Descalsota-Empleo, G.I.; Noraziyah, A.A.S.; Navea, I.P.; Chung, C.; Dwiyanti, M.S.; Labios, R.J.D.; Ikmal, A.M.; Juanillas, V.M.; Inabangan-Asilo, M.A.; Amparado, A.; et al. Genetic Dissection of Grain Nutritional Traits and Leaf Blight Resistance in Rice. *Genes* **2019**, *10*, 30. [CrossRef] [PubMed]
3. Singh, S.P.; Gruissem, W.; Bhullar, N.K. Single genetic locus improvement of iron, zinc and β-carotene content in rice grains. *Sci. Rep.* **2017**, *7*, 6883. [CrossRef] [PubMed]
4. Garg, M.; Sharma, N.; Sharma, S.; Kapoor, P.; Kumar, A.; Chunduri, V.; Arora, P. Biofortified crops generated by breeding; agronomy; and transgenic approaches are improving lives of millions of people around the world. *Front. Nutr.* **2018**, *5*, 12. [CrossRef] [PubMed]
5. Pradhan, S.K.; Pandit, E.; Pawar, S.; Bharati, B.; Chatopadhyay, K.; Singh, S.; Dash, P.; Reddy, J.N. Association mapping reveals multiple QTLs for grain protein content in rice useful for biofortification. *Mol. Genet. Genom.* **2019**, *294*, 963–983. [CrossRef]
6. Kumar, A.K.; Govindaraj, M.; Karthikeyan, A.; Shobhana, V.G.; Warkentin, T.D. Genomics-Integrated Breeding for Carotenoids and Folates in Staple Cereal Grains to Reduce Malnutrition. *Front. Genet.* **2020**, *11*, 414. [CrossRef]
7. Zhu, C.; Kobayashi, K.; Loladze, I.; Zhu, J.; Jiang, Q.; Xu, X.; Liu, G.; Seneweera, S.; Ebi, K.L.; Drewnowski, A.; et al. Carbon dioxide (CO_2) levels this century will alter the protein, micronutrients, and vitamin content of rice grains with potential health consequences for the poorest rice-dependent countries. *Sci. Adv.* **2018**, *4*, eaaq1012. [CrossRef]
8. Pradhan, S.K.; Pandit, E.; Pawar, S.; Naveenkumar, R.; Barik, S.R.; Mohanty, S.P.; Nayak, D.K.; Ghritlahre, S.K.; Rao, D.S.; Reddy, J.N.; et al. Linkage disequilibrium mapping for grain Fe and Zn enhancing QTLs useful for nutrient dense rice breeding. *BMC Plant Biol.* **2020**, *20*, 57. [CrossRef]
9. Sanghamitra, P.; Barik, S.R.; Bastia, R.; Mohanty, S.P.; Pandit, E.; Behera, A.; Mishra, J.; Kumar, G.; Pradhan, S.K. Detection of Genomic Regions Controlling the Antioxidant Enzymes, Phenolic Content, and Antioxidant Activities in Rice Grain through Association Mapping. *Plants* **2022**, *11*, 1463. [CrossRef]
10. Shao, Y.; Jin, L.; Zhang, G.; Lu, Y.; Shen, Y.; Bao, J. Association mapping of grain color, phenolic content, flavonoid content and antioxidant capacity in dehulled rice. *Theor. Appl. Genet.* **2011**, *122*, 1005–1016. [CrossRef]
11. Shao, Y.; Bao, J. Polyphenols in whole rice grain: Genetic diversity and health benefits. *Food Chem.* **2015**, *180*, 86–97. [CrossRef] [PubMed]
12. Panda, S.K. Assay Guided Comparison for Enzymatic and Non-Enzymatic Antioxidant Activities with Special Reference to Medicinal Plant. In *Antioxidant Enzyme*; El-Missiry, A.M., Ed.; IntechOpen: London, UK, 2012; Chapter 15; pp. 381–400. [CrossRef]
13. Govindaraj, M.; Masilamani, P.V.; Albert, A.; Bhaskaran, M. Role of antioxidant in seed quality—A review. *Agric. Rev.* **2017**, *38*, 180–190. [CrossRef]
14. Ferreira, I.C.F.R.; Abreu, R.M.V. Stress oxidativo, antioxidantes e fitoquímicos. *Bioanálise* **2007**, *2*, 32–39.

15. Rossatto, T.; de-Amaral, M.N.; Benitez, L.C.; Vighi, I.L.; Braga, E.; de Magalhães, J.; Maia, A.M.M.; da Silva, P.L. Gene expression and activity of antioxidant enzymes in rice plants, cv. BRS AG, under saline stress. *Physiol. Mol. Biol. Plants* **2017**, *23*, 865–875. [CrossRef] [PubMed]
16. Islam, M.; Begum, H.; Ali, M.; Kamruzzaman, M.; Hoque, S.; Hoque, M. DNA fingerprinting and genetic diversities in some Bangladeshi aus rice (*Oryza sativa* L.) genotypes. *SAARC J. Agric.* **2017**, *15*, 123–137. [CrossRef]
17. Sanghamitra, P.; Sah, R.P.; Bagchi, T.B.; Sharma, S.G.; Kumar, A.; Munda, S.; Sahu, R.K. Evaluation of variability and environmental stability of grain quality and agronomic parameters of pigmented rice (O. sativa L.). *J. Food Sci. Technol.* **2018**, *55*, 879–890. [CrossRef]
18. Mbanjo, E.G.N.; Kretzschmar, T.; Jones, H.; Ereful, N.; Blanchard, C.; Boyd, L.A.; Sreenivasulu, N. The Genetic Basis and Nutritional Benefits of Pigmented Rice Grain. *Front. Genet.* **2020**, *11*, 229. [CrossRef]
19. Xu, F.; Jinsong, B.; Tae-Sung, K.; Yong-Jin, P. Genome-wide Association Mapping of Polyphenol Contents and Antioxidant Capacity in Whole-Grain Rice. *J. Agric. Food Chem.* **2016**, *64*, 4695–4703. [CrossRef]
20. Zhai, S.N.; Xia, X.C.; He, Z.H. Carotenoids in Staple Cereals: Metabolism, Regulation, and Genetic Manipulation. *Front. Plant Sci.* **2016**, *7*, 1197. [CrossRef]
21. Ashraf, H.; Murtaza, I.; Nazir, N.; Wani, A.B.; Naqash, S.; Husaini, A.M. Nutritional profiling of pigmented and scented rice genotypes of Kashmir Himalayas. *J. Pharmacogn. Phytochem.* **2017**, *6*, 910–916.
22. Petroni, K.; Landoni, M.; Tomay, F.; Calvenzani, V. Proximate composition, polyphenol content and anti-inflammatory properties of white and pigmented Italian rice varieties. *Univ. J. Agric. Res.* **2017**, *5*, 312–321. [CrossRef]
23. Furukawa, T.; Maekawa, M.; Oki, T.; Suda, I.; Iida, S.; Shimada, H.; Takamure, I.; Kadowaki, K. The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. *Plant J. Cell Mol. Biol.* **2006**, *49*, 91–102. [CrossRef] [PubMed]
24. Sweeney, M.T.; Thomson, M.J.; Pfeil, B.E.; Mccouch, S. Caught red-handed: Rc encodes a basic Helix-Loop-Helix protein conditioning red pricarp in rice. *Plant Cell.* **2006**, *18*, 283–294. [CrossRef] [PubMed]
25. Dong, Y.J.; Xu, J.L.; Xiao, K.; Zhang, Y.J.; Zhang, J.Z.; Luo, L.J.; Mastuo, M. Genomic regions associated with the degree of red coloration in pericarp of rice (*Oryza sativa L.*). *J. Cereal Sci.* **2008**, *48*, 556–560. [CrossRef]
26. Yoshimura, A.; Ideta, O.; Iwata, N. Linkage map of phenotype and RFLP markers in rice. *Plant Mol. Biol.* **1997**, *35*, 49–60. [CrossRef] [PubMed]
27. Kim, B.G.; Kim, J.H.; Min, S.Y.; Shin, K.H.; Kim, J.H.; Kim, H.Y.; Ryu, S.N.; Ahn, J.H. Anthocyanin content in rice is related to repeat protein from Medicagotruncatula is necessary for tissue-specific anthocyanin and proanthocyanidin biosynthesis but not for trichome development. *Plant Physiol.* **2007**, *151*, 1114–1129.
28. Sun, X.; Zhang, Z.; Chen, C.; Wu, W.; Ren, N.; Jiang, C.; Yu, J.; Zhao, Y.; Zheng, X.; Yang, Q.; et al. The C–S–A gene system regulates hull pigmentation and reveals evolution of anthocyanin biosynthesis pathway in rice. *J. Exp. Bot.* **2018**, *69*, 1485–1498. [CrossRef] [PubMed]
29. Oikawa, T.; Maeda, H.; Oguchi, T.; Yamaguchi, T.; Tanabe, N.; Ebana, K.; Yano, M.; Ebitani, T.; Izawa, T. The birth of a black rice gene and its local spread by introgression. *Plant Cell* **2015**, *27*, 2401–2414. [CrossRef]
30. Yang, X.; Xia, X.; Zeng, Y.; Nong, B.; Zhang, Z.; Wu, Y.; Xiong, F.; Zhang, Y.; Liang, H.; Deng, G.; et al. Identification of candidate genes for gelatinization temperature, gel consistency and pericarp color by GWAS in rice based on SLAF-sequencing. *PLoS ONE* **2018**, *13*, e0196690. [CrossRef]
31. Shao, Y.; Tang, F.; Huang, Y.; Xu, F.; Chen, Y.; Tong, C.; Chen, H.; Bao, J. Analysis of genotype × environment interactions for polyphenols and antioxidant capacity of rice by association mapping. *J. Agric. Food Chem.* **2014**, *62*, 5361–5368. [CrossRef]
32. Tan, Y.F.; Sun, M.; Xing, Y.Z.; Hua, J.P.; Sun, X.L.; Zhang, Q.F.; Corke, H. Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. *Theor. Appl. Genet.* **2001**, *103*, 1037–1045. [CrossRef]
33. Jin, L.; Xiao, P.; Lu, Y.; Shao, Y.F.; Shen, Y.; Bao, J.S. Quantitative trait loci for brown rice color, total phenolics and, flavonoid contents and antioxidant capacity in rice grain. *Cereal Chem.* **2009**, *86*, 609–615. [CrossRef]
34. Kato, T.; Matsukawa, T.; Horibata, A. Quantitative trait loci responsible for the difference in γ-oryzanol content in brown rice between japonica-type and indica-type rice cultivars. *Plant Prod. Sci.* **2017**, *20*, 459–466. [CrossRef]
35. Sahoo, S.; Sanghamitra, P.; Nanda, N.; Pawar, S.; Pandit, E.; Bastia, R.; Muduli, K.C.; Pradhan, S.K. Association of molecular markers with physio-biochemical traits related to seed vigour in rice. *Physiol. Mol. Biol. Plants* **2020**, *26*, 1989–2003. [CrossRef]
36. Shih, C.H.; Chu, H.; Tang, L.K.; Sakamoto, W.; Maekawa, M.; Chu, I.K.; Wang, M.; Lo, C. Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta* **2008**, *228*, 1043–1054. [CrossRef]
37. Cai, Z.; He, F.; Feng, X.; Liang, T.; Wang, H.; Ding, S.; Tian, X. Transcriptomic Analysis Reveals Important Roles of Lignin and Flavonoid Biosynthetic Pathways in Rice Thermotolerance During Reproductive Stage. *Front. Genet.* **2020**, *11*, 562937. [CrossRef]
38. Bai, C.; Capell, T.; Berman, J.; Medina, V.; Sandmann, G.; Christou, P.; Zhu, C. Bottlenecks in carotenoid biosynthesis and accumulation in rice endosperm are influenced by the precursor–product balance. *Plant Biotechnol. J.* **2016**, *14*, 195–205. [CrossRef]
39. You, M.K.; Lee, Y.J.; Kim, J.K.; Baek, S.A.; Jeon, Y.A.; Lim, S.H.; Ha, S.H. The organ-specific differential roles of rice DXS and DXR, the first two enzymes of the MEP pathway, in carotenoid metabolism in *Oryza sativa* leaves and seeds. *BMC Plant Biol.* **2020**, *20*, 167. [CrossRef]
40. Tian, Y.; Wang, B.; Peng, R.; Xu, J.; Li, T.; Fu, X.; Xiong, A.; Gao, J.; Yao, Q. Enhancing carotenoid biosynthesis in rice endosperm by metabolic engineering. *Plant Biotechnol. J.* **2019**, *17*, 849–851. [CrossRef]

41. Xia, D.; Hao, Z.; Yipei, W.; Pingbo, L.; Pei, F.; Bian, W.; Yuqing, H. How rice organs are colored: The genetic basis of anthocyanin biosynthesis in rice. *Crop J.* **2021**, *9*, 598–608. [CrossRef]
42. Goufo, P.; Trindade, H. Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Sci. Nutr.* **2014**, *2*, 75–104, PMCID:PMC3959956. [CrossRef] [PubMed]
43. Mackon, E.; Mackon, G.J.D.E.; Ma, Y.; Kashif, M.H.; Ali, N.; Usman, B.; Liu, P. Recent Insights into Anthocyanin Pigmentation, Synthesis, Trafficking, and Regulatory Mechanisms in Rice (*Oryza sativa* L.) Caryopsis. *Biomolecules* **2021**, *11*, 394. [CrossRef] [PubMed]
44. Huchzermeyer, B.; Menghani, E.; Khardia, P.; Shilu, A. Metabolic Pathway of Natural Antioxidants, Antioxidant Enzymes and ROS Providence. *Antioxidants* **2022**, *11*, 761. [CrossRef] [PubMed]
45. Pandit, E.; Tasleem, S.; Barik, S.R.; Mohanty, D.P.; Nayak, D.K.; Mohanty, S.P.; Das, S.; Pradhan, S.K. Genome-wide association mapping reveals multiple QTLs governing tolerance response for seedling stage chilling stress in indica rice. *Front. Plant Sci.* **2017**, *8*, 552. [CrossRef] [PubMed]
46. Pawar, S.; Pandit, E.; Mohanty, I.C.; Saha, D.; Pradhan, S.K. Population genetic structure and association mapping for iron toxicity tolerance in rice. *PLoS ONE* **2021**, *16*, e0246232. [CrossRef] [PubMed]
47. Davis, B.H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*, 2nd ed.; Goodwin, T.W., Ed.; Academic Press Inc.: London, UK, 1976; pp. 38–165.
48. Madamanchi, N.R.; Donahue, J.L.; Cramer, C.L.; Alscher, R.G.; Pedersen, K. Differential response of Cu, Zn SOD in two pea cultivars during a short term exposure to SO2. *Plant Mol. Biol.* **1994**, *26*, 95–103. [CrossRef]
49. Fuleki, T.; Francis, F.J. Quantitative methods for anthocyanins, extraction and determination of total anthocyanin in cranberries. *J. Food Sci.* **1968**, *33*, 72–77. [CrossRef]
50. Bucci, R.; Magri, A.D.; Magri, A.L.; Marini, F. Comparison of three spectrophotometric methods for the determination of gammaoryzanol in rice bran oil. *Anal. Bioanal. Chem.* **2003**, *375*, 1254–1259. [CrossRef]
51. Eberhardt, M.V.; Lee, C.Y.; Liu, R.H. Antioxidant activity of fresh apples. *Nature* **2000**, *405*, 903–904. [CrossRef]
52. Serpen, A.; Gokmen, V.; Pellegrini, N.; Fogliano, V. Direct measurement of the total antioxidant capacity of cereal products. *J. Cereal Sci.* **2008**, *48*, 816–820. [CrossRef]
53. Murray, M.G.; Thompson, W.F. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* **1980**, *8*, 4321–4325. [CrossRef] [PubMed]
54. Barik, S.R.; Pandit, E.; Pradhan, S.K.; Singh, S.; Mohapatra, T. QTL mapping for relative water content trait at reproductive stage drought tolerance in rice. *Ind. J. Genet. Plant Breed.* **2018**, *78*, 401–408.
55. Barik, S.R.; Pandit, E.; Pradhan, S.K.; Mohanty, S.P.; Mohapatra, T. Genetic mapping of morpho-physiological traits involved during reproductive stage drought tolerance in rice. *PLoS ONE* **2009**, *14*, e0214979. [CrossRef] [PubMed]
56. Pradhan, S.K.; Pandit, E.; Pawar, S.; Baksh, S.Y.; Mukherjee, A.K.; Mohanty, S.P. Development of flash-flood tolerant and durable bacterial blight resistant versions of mega rice variety 'Swarna' through marker-assisted backcross breeding. *Sci. Rep.* **2019**, *9*, 12810. [CrossRef] [PubMed]
57. Liu, K.; Muse, S.V. Power Marker: An integrated analysis environment for genetic marker analysis. *Bioinformatics* **2005**, *21*, 2128–2129. [CrossRef] [PubMed]
58. Pritchard, J.K.; Stephens, M.; Donnelly, P. Inference of population structure using multilocus genotype data. *Genetics* **2000**, *155*, 945–959. [CrossRef] [PubMed]
59. Evanno, G.; Regnaut, S.; Goudet, J. Detecting the number of clusters of individualsusingthe software STRUCTURE: A simulation study. *Mol. Ecol.* **2005**, *14*, 2611–2620. [CrossRef] [PubMed]
60. Earl, D.A.; Vonholdt, B.M. Structure Harvester: A website and program for visualizing STRUCTURE output and implementing the Evannomethod. *Conserv. Genet. Resour.* **2012**, *4*, 359–361. [CrossRef]
61. Nei, M. Genetic distance between populations. *Am. Nat.* **1972**, *106*, 283–292. [CrossRef]
62. Perrier, X.; Jacquemoud-Collet, J.P. *DARwin Software*; Version 6.0.018; CIRAD: Montpellier, France, 2006; Available online: http://darwin.cirad.fr/darwin (accessed on 24 August 2022).
63. Pandit, E.; Panda, R.K.; Sahoo, A.; Pani, D.R.; Pradhan, S.K. Genetic relationship and structure analyses of root growth angle for improvement of drought avoidance in early and mid-early maturing rice genotypes. *Rice Sci.* **2020**, *27*, 124–132. [CrossRef]
64. Pradhan, S.K.; Barik, S.R.; Sahoo, A.; Mohapatra, S.; Nayak, D.K.; Mahender, A.; Meher, J.; Anandan, A.; Pandit, E. Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. *PLoS ONE* **2016**, *11*, 123. [CrossRef] [PubMed]
65. Bradbury, P.J.; Zhang, Z.; Kroon, D.E.; Casstevens, T.M.; Ramdoss, Y.; Buckler, E.S. TASSEL: Software for association mapping of complex traits in diverse samples. *Bioinform. Appl. Note* **2007**, *23*, 2633–2635. [CrossRef] [PubMed]
66. Sanghamitra, P.; Bagchi, T.B.; Sharma, S.G.; Sarkar, S.; Basak, N. Characterization of red and purple-pericarp rice (*Oryzasativa* L.) based on physico-chemical and antioxidative properties of grains. *Oryza* **2017**, *54*, 57–64.
67. Sanghamitra, P.; Nanda, N.; Barik, S.; Sahoo, S.; Pandit, E.; Bastia, R.; Bagchi, T.B.; Pradhan, S.K. Genetic structure and molecular markers-trait association for physiological traits related to seed vigour in rice. *Plant Gene* **2021**, *28*. [CrossRef]
68. Arjun, P.; Wagh, M.; Pawar, S.; Mohanty, I.C.; Pandit, E.; Meher, J.; Pradhan, S.K. Genetic Structure and Markers—Trait Association Analyses for Fe-Toxicity Tolerance, Grain-Fe Content and Yield Component Traits in Rice. *J. Plant Biol. Crop Res.* **2020**, *3*, 1022.

69. Patra, B.C.; Dhua, S.R. Agro-morphological diversity scenario in upland rice germplasm of Jeypore tract. *Genet. Resour. Crop Evol.* **2003**, *50*, 825–828. [CrossRef]
70. Barik, S.R.; Pandit, E.; Sanghamitra, P.; Mohanty, S.P.; Behera, A.; Mishra, J.; Nayak, D.K.; Bastia, R.; Moharana, A.; Sahoo, A.; et al. Unraveling the genomic regions controlling the seed vigour index, root growth parameters and germination per cent in rice. *PLoS ONE* **2022**, *17*, e0267303. [CrossRef]
71. Huang, X.; Zhao, Y.; Wei, X.; Li, C.; Wang, A.; Zhao, Q.; Li, W.; Guo, Y.; Deng, L.; Zhu, C.; et al. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat. Genet.* **2011**, *44*, 32–39. [CrossRef] [PubMed]
72. Oselebe, H.O.; Ogah, O.; Odo, M.I.; Ogbu, K.I. Determination of Phytochemical and Antioxidant Properties of Some Rice Varieties and Hybrids Grown in Ebonyi State, Nigeria. *Nig. J. Biotechnol.* **2013**, *26*, 60–67.
73. Saini, A.; Rohila, J.S.; Govindan, G.; Li, Y.F.; Sunkar, R. Splice Variants of Superoxide Dismutases in Rice and Their Expression Profiles under Abiotic Stresses. *Int. J. Mol. Sci.* **2021**, *22*, 3997, PMCID:PMC8068833. [CrossRef] [PubMed]
74. Reddy, A.R. Genetic and molecular analysis of the anthocyanin pigmentation pathway in rice. In *Rice Genetics III. Proceedings of the Third International Rice Genetics Symposium*; Khush, G.S., Ed.; International Rice Research Institute: Manila, Philippines, 2008; pp. 341–352.
75. Reddy, A.M.; Reddy, V.S.; Scheffler, B.E.; Wienand, U.; Reddy, A.R. Novel transgenic rice overexpressing anthocyanidin synthase accumulates a mixture of flavonoids leading to an increased antioxidant potential. *Metab. Eng.* **2007**, *9*, 95–111. [CrossRef] [PubMed]
76. Zhang, F.; Yang, L.; Huang, W.; Luo, X.; Xie, J.; Hu, B.; Chen, Y. Flavonoid Metabolic Profiles and Gene Mapping of Rice (*Oryza sativa* L.) Purple Gradient Grain Hulls. *Rice* **2022**, *15*, 43. [CrossRef] [PubMed]